"A STUDY OF HEMATOLOGICAL INDICES AND HISTOGRAM FINDINGS IN CLINICALLY SUSPECTED MALARIAL FEVER IN COMPARISON WITH GOLD STANDARD TEST FOR MALARIA FOR RAPID SCREENING"

Dissertation submitted in

partial fulfilment of the requirements for the degree of

M.D. PATHOLOGY

BRANCH – III

INSTITUTE OF PATHOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADUDR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

MAY 2020

CERTIFICATE

This is to certify that this Dissertation entitled **"A STUDY OF HEMATOLOGICAL INDICES AND HISTOGRAM FINDINGS IN CLINICALLY SUSPECTED MALARIAL FEVER IN COMPARISON WITH GOLD STANDARD TEST FOR MALARIA FOR RAPID SCREENING"**is the bonafide original work of **DR.SRIDHAR .S** in partial fulfilment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in May 2020.

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DECLARATION

I, **DR.SRIDHAR S,** solemnly declare that the dissertation entitled **"A STUDY OF HEMATOLOGICAL INDICES AND HISTOGRAM FINDINGS IN CLINICALLY SUSPECTED MALARIAL FEVER IN COMPARISON WITH GOLD STANDARD TEST FOR MALARIA FOR RAPID SCREENING"**is the bonafide work done by me at the Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. BHARATHI VIDHYA JAYANTHI, M.D.,** Professor of Pathology, Institute of Pathology and **DR.RAMYA, M.D.,** Assistant Professor of Pathology, Institute of Pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **DR.SRIDHAR .S**

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CERTIFICATE OF APPROVAL

To

Dr. S. SRIDHAR
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Dear Dr.S. SRIDHAR

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY OF HEMATOLOGICAL INDICES AND HISTOGRAM FINDINGS IN CLINICALLY SUSPECTED MALARIAL FEVER IN COMPARISON WITH GOLD STANDARD TEST FOR MALARIA FOR RAPID SCREENING" - NO.09032018**

The following members of Ethics Committee were present in the meeting held on **27.03.2018** conducted at Madras Medical College, Chennai 3

1. Prof.P.V.Jayashankar :Chairperson
2. Prof.R.Jayanthi,MD.,FRCP(Glasg) Dean,MMC,Ch-3 : Deputy Chairperson
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13. Tmt.Arnold Saulina, MA.,MSW., :Social Scientist
14. Thiru K.Ranjith, Ch- 91 : Lay Person

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

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ABBREVIATION

WBC	:	White blood cells
RBC	:	Red Blood cells
HGB	:	Hemoglobin
HCT	:	Hematocrit
MCV	:	Mean corpuscular volume
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Hemoglobin Concentration
PLT	:	Platelets
RDW- SD	:	Red Cell Distribution Width
PDW	:	Platelet Distribution Width
MPV	:	Mean Platelet Volume
P-LCR	:	Platelet Large cell ratio
PCT	:	Plateletcrit
NRBC %	:	Nucleated RBC %
NEUT%	:	Neutrophil percentage
LYMP%	:	Lymphocyte Percentage
MONO%	:	Monocyte Percentage
EO%	:	Eosinophil percentage
Baso%	:	Basophil percentage.

CONTENTS

INTRODUCTION

INTRODUCTION

Malaria is a potentially life threatening disease caused by parasites (Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale that are transmitted through the bite of infected Anopheles mosquitoe.[1]WHO estimates that India accounts for 89 % malaria cases in South-East Asia. Increased malaria prevention and control measures have dramatically reduced malaria across many countries, including India. The incidence rate of malaria is estimated to have decreased by 18% globally between 2010 and 2016. The Government of India launched the National Framework for Malaria Elimination 2016-2030 in February 2016 and the National Strategic Plan for Malaria Elimination 2017-2022 in July 2017 with WHO support.India has a vision of a malaria free country by 2027 and elimination by 2030.

AS PER NATIONAL HEALTH MISSION TAMILNADU,

In Tamil Nadu, Malaria is confined to some of the Urban, Coastal and Riverine areas such as Corporation of Chennai, Ramanathapuram, Paramakudi, Thoothukudi, Kanyakumari, Krishnagiri, Dharmapuri and Thiruvannamalai.[2] Chennai being endemic to this parasite hence a study of this sort receives attention.

WHO recommends microscopy based (Gold standard for malaria) diagnosis in all patients suspected of malaria before starting treatment.

Malaria rapid diagnostic tests (RDTs) have been used in the rapid screening in primary level health care and field level screening since it is relatively simple to perform and interpret even by unskilled paramedical and village level staffs.[3]

The MHFW Government of India, has prohibited the use of the Antibody Detecting Rapid Diagnostic Tests (ADRDT) for routine diagnosis of malaria from 2017[4] due to

- Low sensitivity in detecting asymptomatic patients particularly in low parasitemias
- Cross reactions to autoantibodies in the patient serum,
- False positivity (gametocytemia, persistent asexual-stage parasitemia below microscopy detection limit)
- False negativity (genetic deletion or mutation of hrp-2 antigen

Due to the above pitfalls with RDT there is a immense need in finding a alternate diagnostic test which performs as well like the Gold standard test (MICROSCOPY)for malaria for rapid screening for malaria in remote areas

Hematological abnormalities are considered a hallmark of malaria various studies have confirmed the same. Malaria causes various immune response like innate and aquired immunity which involves both cellular and humoral immunity from the host adding on to its pathogenisity .These CBC parameters get altered due to the complex pathogenicity of the plasmodium species in its asexual erythrocytic stage infecting RBC. Hence automated

analyzers generated reports with altered CBC parameters and their histogram flags[5] can be used as a diagnostic clue to malaria at the primary health care level even by semi skilled heath workers in remote areas at par with thick and thin blood smear examination by microscopy which is gold standard test for diagnosing malaria

. Malaria diagnosis with PCR , flow cytometry based hematology analyzers could become an important adjuvant diagnostic tool in routine laboratory work up of febrile patients in malaria endemic [6]

However the high cost and expert skill required to operate these machines in the primary care level rules out its practical feasibility in using these devices both in primary health centre and mass field screening in India.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

To evaluate the usefulness of automated cell counters analyzing the hematological parameters, their histograms, and the flags generated from the blood samples of Clinically suspected malaria cases in comparison with gold standard test for malaria for rapid screening.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

EPIDEMIOLOGY

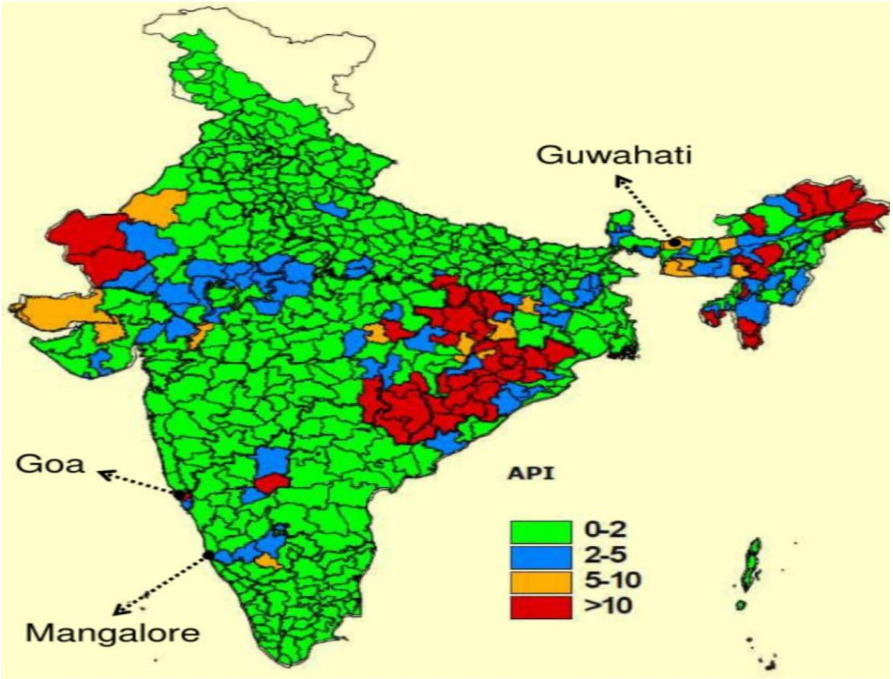
The World Malaria Report 2018 estimates that there were 219 million cases and 435 000 deaths in 2017 globally. Nearly half of the world's population is at risk of malaria. In 2017, nearly 70% of all malaria cases and deaths were concentrated in 11 countries: 10 in Africa and India. India has the fourth highest malaria burden in the world.

World Malaria Report 2018 has documented the remarkable decline in malaria cases and deaths in India.

As per the report, 24% reduction in cases of malaria was recorded in 2017 compared to 2016. In 2018, there has been a 52% decline in cases compared to 2017.

High endemic states of Odisha, Chhattisgarh, Jharkhand, Madhya Pradesh and Meghalaya, which accounted for nearly 77% cases of malaria, have shown a sharp decline. [6]

FIG 1MALARIA ENDEMIC AREAS IN INDIA[6]



Tamil Nadu comes under the category of API (Annual Parasitic Insex) of less than 1 case per 1000 population at risk where the infection is in the elimination Phase.[6]

In the context of tamilnadu , chennai endemic region for the incidence and prevalence of malaria.Hence a study from this region gains importance.

The proportion of plasmodium vivax and plasmodium falciparum varies in different part of india.

Plasmodium falciparum accounts for 30-90% of infections in forested areas inhabited by ethnic tribes and <10% of cases in mostly indo .gangetic plains , northen hilly stations, north western India and Tamilnadu .In our study only one

case was reported as plasmodium falciparum out of 60 smear positive cases for malaria.Rest 59 cases were Plasmodium vivax positive.

FIG2. TREND OF MALARIAL INFECTION IN THE PAST TWO DECADES.(2001-2018 TILL JULY)[7]

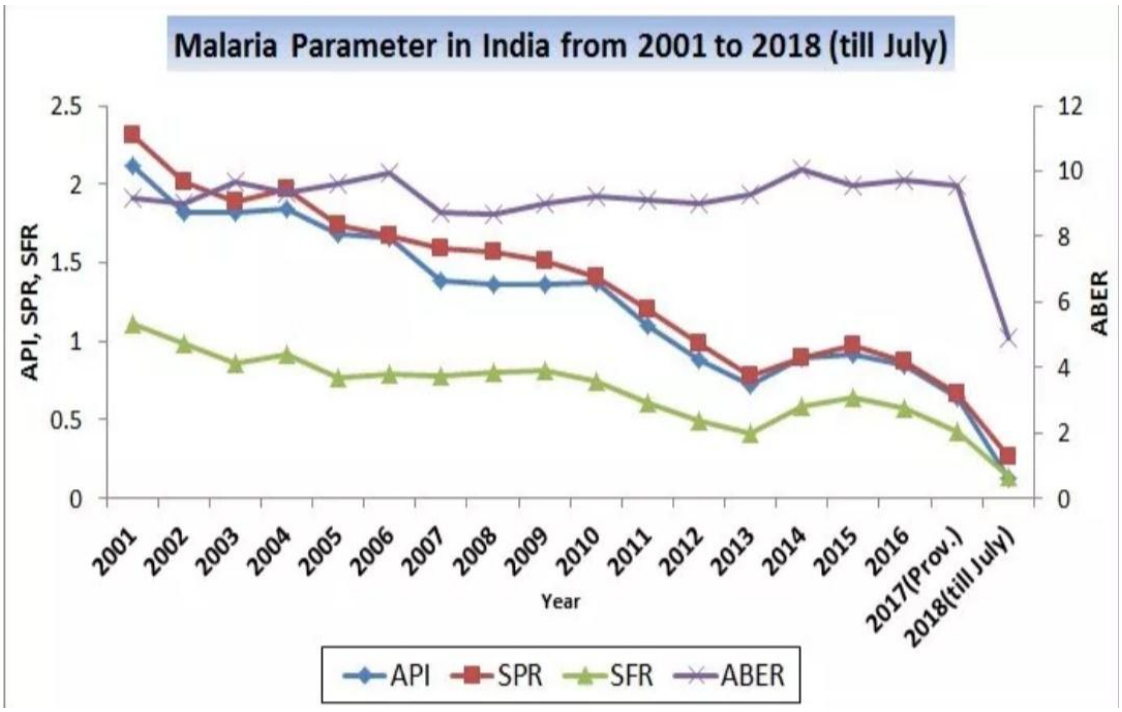


TABLE 1. EPIDEMIOLOGICAL SITUATION REPORT 2015 AND 2016 (ALL OVER INDIA AND TAMILNADU)[7]

S No	Year	All India/Tamil Nadu	+VE cases P.V	+VE cases P.F	Total	Pf%	ABER	API	SPR	AFI	SFR	Death
1	2015	All India	390440	778821	1169261	66.1	9.58	0.92	0.97	0.62	0.64	384
2	2015	Tamil Nadu	5232	355	5587	6.35	11.65	0.07	0.06	0	0	0
3	2016	All India	374511	716213	1090724	65.6	9.74	0.85	0.87	0.56	0.57	331
4	2016	Tamil Nadu	4099	242	4341	5.57	10.82	0.06	0.05	0	0	0

TABLE 2 RECENT DATA OF CASE REPORTED AND DEATH REPORTED IN ALL INDIA AND TAMILNADU.(SOURCE NVDCP) [8]

Year	Number of cases	All India/Tamilnadu	Deaths
2017	844558	All India	194
2017	5444	Tamilnadu	0
2018	399134	All India	85
2019	3762	Tamilnadu	0

With the above data taken from national framework for malaria elimination in India it is very clear that the incidence and prevalence of malaria has gone down in Tamilnadu when compared to the indian population as whole from different states and union territories of India.[7]

The incidence and prevalence of plasmodium falciparum is very much reduced in Tamilnadu when compared to All India data.

In the current institutional study carried out in Madras medical college Rajeev Gandhi Hospital between 1st april2018-1st October 2019.

Total cases reported positive and documented during the study were 60 which includes 59cases from plasmodium vivax and only one case of falciparum malaria.

VECTOR FOR MALARIA TRANSMISSION

Low prevalence of falciparum malaria in our study is documented which is supported by national statistics.Malaria in India is mainly caused by two major malaria parasites namelyPlasmodium falciparum and *P*lasmodium Vivax.

Cases of malaria from Plasmodium ovale and Plasmodium malariae have also been reported from some parts of our country .

P. falciparum (Pf) and P. vivax (Pv) are the most common species causing malaria in India.

P. vivax is more prevalent in the plains, *P. falciparum* predominates in forested and peripheral areas.

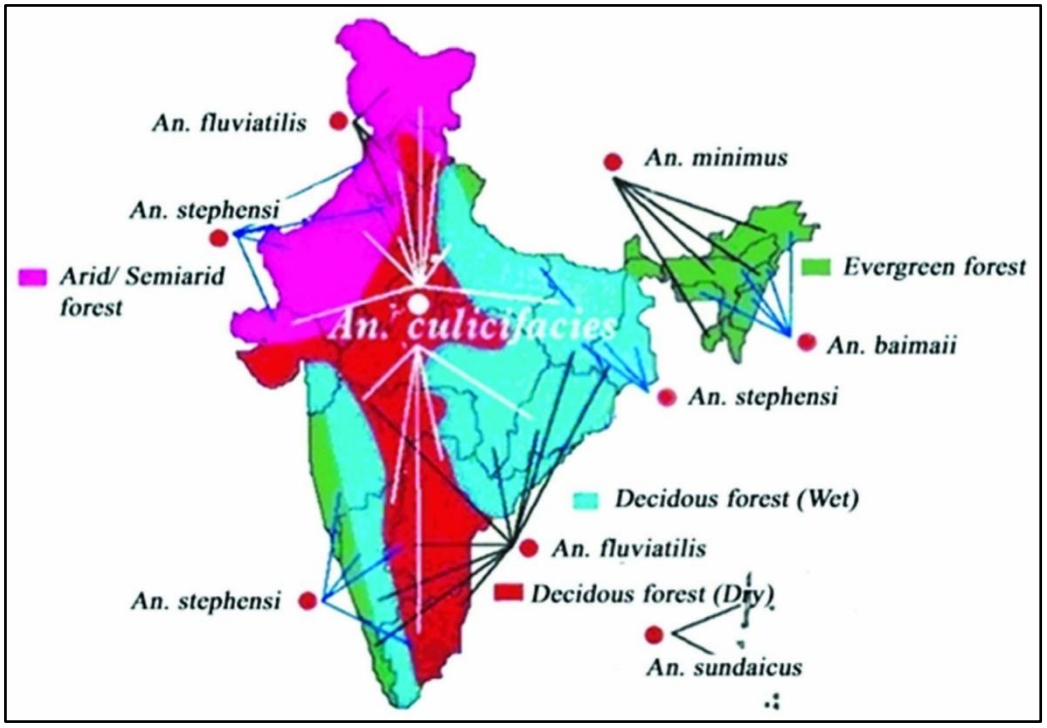
The disease is transmitted by nine *Anopheline* species out of which the six primary vectors are Anopheles culicifacies, Anopheles stephensi, Anopheles dirus, Anopheles fluviatilis, Anopheles minimus and Anopheles epiroticus .

Out of these An. culicifacies and An. Stephensi are common species in south india especially tamilnadu.

1. An. culicifacies is widespread in peninsular India. It is the main vector of malaria in rural plains areas and peri-urban areas. It is found in a variety of natural and man-made breeding sites.

2. An. stephensi,shares breeding sites with An. culicifacies are seen growing in artificial containers, and is responsible for malaria in urban and industrial areas.

FIG 3.DISTRIBUTION OF MALARIAL VECTOR ACROSS INDIA.[6]



Anophilus culicifacies and anophelous stephensi is common vector in Tamilnadu. During the months from June to September, the country experiences the monsoon season characterized by heavy rains across different states of the country. It is during these months that maximum transmission of malaria takes place. In the immediate post-monsoon period from October to December, collection of rainwater in pits and puddles promotes mosquito breeding and subsequently the transmission of malaria.

LIFE CYCLE OF MALARIAL PARASITES AND ITS PATHOGENESIS

The life cycle of malaria parasites is complex like any other parasite it needs specialized protein expression for survival in both the invertebrate and vertebrate hosts.

These proteins are required for both intracellular (erythrocytic stage)and extracellular survival (non erythrocytic stage) of the parasite and the evasion of host immune responses like any other parasite.[8]

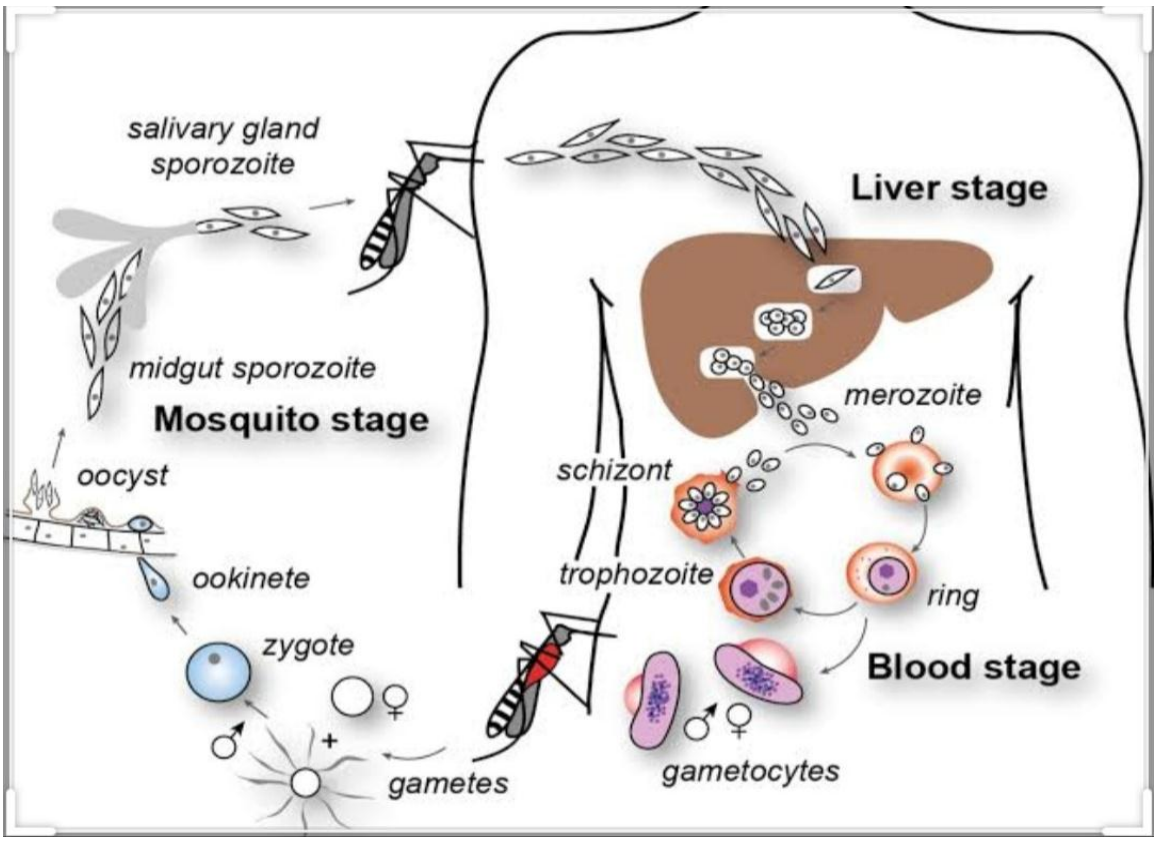
The parasite is injected into the human host from the of the vector female annophiline mosquito -

The Plasmodium falciparum and Plasmodium malariae sporozoites trigger immediate schizogony, the Plasmodium ovale and Plasmodium vivax sporozoites may either trigger immediate schizogony or lead to delayed schizogony as they pass through the hypnozoite stage mentioned above.

The life cycle of the malaria parasite is shown in FIG 4 and can be divided into several stages, starting with sporozoite entry into the bloodstream.

FIG 4 LIFE CYCLE OF MALARIAL PARASITE

(example of falciparum malaria)



JOURNAL OF CELL BIOLOGY [9]

Once in the human bloodstream,P. *falciparum* sporozoites reach the liver and penetrate the liver cells (hepatocytes) where they remain for 9–16 undergo asexual replication known as exo-erythrocytic schizogony.

The receptors on sporozoites responsible for hepatocyte invasion are mainly the thrombospondin domains on the circumsporozoite protein and on thrombospondin-related adhesive protein. These domains specifically bind to heparan sulfate proteoglycans on the hepatocytes [10]

Each sporozoite gives rise to tens of thousands of merozoites inside the hepatocyte and each merozoite can invade a red blood cell (RBC) on release from the liver.

Hepatocyte-derived merosomes appear to act as shuttles that ensure the protection of parasites from the host immune system and the release of viable merozoites directly into the circulation [11]

Erythrocytic schizogony

Merozoites enter erythrocytes by a complex invasion process which is be divided into four phases:

(a) initial recognition and reversible attachment of the merozoite to the erythrocyte membrane;
(b) Followed by irreversible attachment and the release of substances from the rhoptry and microneme organelles, leading to formation of the parasitophorous vacuole;
(c) Invagination of the erythrocyte membrane around the merozoite accompanied by removal of the merozoite's surface coat; and
(d) resealing of the parasitophorous vacuole and erythrocyte membranes after completion of merozoite invasion [12][13]

Asexual division starts inside the erythrocyte and the parasites develop through different stages .

The early trophozoite is the ‘ring form’, because of its characteristic morphology (FIG4]

Trophozoite enlargement is accompanied by highly active metabolism, which includes glycolysis of large amounts of imported glucose, the ingestion of host cytoplasm and the proteolysis of hemoglobin into constituent amino acids.

Malaria parasites cannot degrade the heme by-product and free heme is potentially toxic to the parasite

During hemoglobin degradation, most of the liberated heme is polymerized into hemozoin (malaria pigment), a crystalline substance that is stored within the food vacuoles .

The end of this trophic stage is marked by multiple rounds of nuclear division resulting in the formation of schizonts [FIG 4]

Each mature schizont contains around 20 merozoites and are released after lysis of the RBC to invade further uninfected RBCs.

The lysis of RBC released TNF and other pyrogenic cytokines responsible for the fever episode.

This release of merozoites causes clinical manifestation of the malarial fever with cl with chills and rigors.

This repetitive intraerythrocytic cycle of invasion–multiplication–release–invasion continues, taking about 48 falciparum P., ovale P. and vivax P. h in infections and 72 malariae in P. infection. which causes the classical fever with chills and rigors in malaria as intermittent fever .

The merozoites are released at approximately the same time of the day. A number of specific ligand–receptor interactions have been identified as involved in invasion and it has been reported that genetic disruption of any one of these results in a shift to using other pathways [14] [15]

The *falciparum* *P.* genome sequence, completed in 2002, indicates that several of the molecules involved in invasion are members of larger gene families[16] [17].

Merozoite surface proteins (MSP−1 to MSP−4) are an important class of integral membrane proteins identified on the surface of developing and free merozoites. These are involved in the initial recognition of the erythrocytes via interactions with sialic acid residues and are likely to be important for invasion because antibodies directed against these proteins can block this process

Erythrocyte binding antigen75 (EBA-175) is a P.*falciparum* *antigen* that binds the major glycoprotein (glycophorin) found on human erythrocytes on invasion.

The structure of EBA-175 has striking similarities with the Duffy antigen-binding proteins of p. *vivax* that are essential for successful invasion by this species.

After invasion, the principal parasite ligand known as (PfEMP1), which is encoded by a multigene family termed erythrocyte membrane protein *var*, is expressed at the surface of the infected RBC in case of faciparum infection[18,19]

The extensive diversity in the *var* gene family is mainly responsible for the evasion of specific immune responses and many of these genes are expressed in the parasite population, but at any given time during an infection, parasites within infected cells express only a single *var* gene [20,21,22]

In a recent study, a specific epigenetic mark associated with the silenced*var* genes has been identified and it has been shown that the persistence of this mark provides advantages to the parasite in pathogenesis and immune evasion[23]

A small proportion of the merozoites in the red blood cells eventually differentiate to produce micro- and macrogametocytes (male and female, respectively), which have no further activity within the human host [FIG.4]

These gametocytes are essential for transmitting the infection to new hosts through female *Anopheles* mosquitoes. Normally, a variable number of cycles of asexual erythrocytic schizogony occur before any gametocytes are produced.

Falciparum being more lethal with severe parasitic load along with high population of gametes which causes transmission of the disease to the close contact when the same mosquito bites during its blood meal.

Sexual phase in the mosquito (sporogony)

A mosquito taking a blood meal on an infected individual may ingest these gametocytes into its midgut, where macrogametocytes form macrogametes and exflagellation of microgametocytes produces microgametes.

These gametes fuse, undergo fertilization and form a zygote. This transforms into an ookinete, which penetrates the wall of a cell in the midgut and develops into an oocyst .

In a recent study, it has been shown that gamete surface antigen Pfs230 mediates human RBC binding to exflagellating male parasites to form clusters termed exflagellation centers, from which individual motile microgametes are released.

This protein thus plays an important role in subsequent oocyst development, which is a critical step in malaria transmission [24]

Sporogony within the oocyst produces many sporozoites and when the oocyst ruptures, they migrate to the salivary glands for onward transmission into another host .

FIG 5 : MORPHOLOGY OF VARIOUS SPECIES OF MALARIAL PARASITE

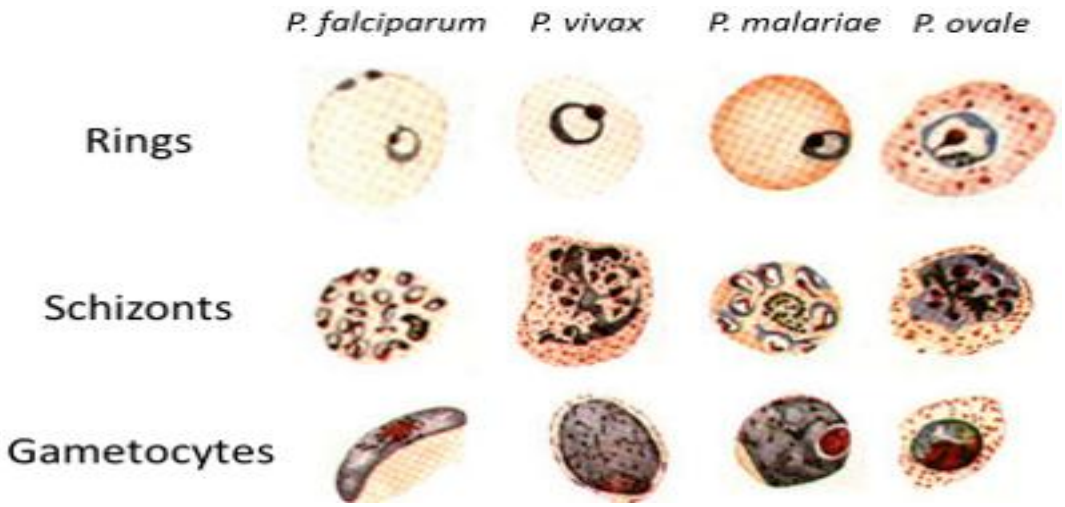


TABLE 3.IDENTIFICATION FOR DIFFERENT SPECIES OF PLASMODIUM IN MICROSCOPY

RBC Nature	**P.Vivax**	**P.Falciparum**	**P.Malarie**	**P.Ovale**
Enlarged ,Pale	**present**	absent	**present**	Absent
Oval , fimbriated	Absent	absent	Absent	**Present**
Schuffner dots	**present**	Absent	Absent	**present**
Maurer dots	Absent	**present**	Absent	Absent
Parasite nature				
All stages of parasite seen	**present**	Absent	**present**	**present**
Band forms	Absent	absent	**present**	Absent
Double infections	Absent	**Present**	Absent	Absent
Double chromatin dots	Absent	**present**	Absent	Absent
Banana shaped gametes	Absent	**present**	absent	absent

DIAGNOSIS OF MALARIA WHO APPROVED METHODS AND PIT FALLS OF EACH.

Currently available techniques

1) Microscopy
2) Immunological Techniques–RDT
3) Molecular Techniques.

MICROSCOPY - GOLD STANDARD FOR MALARIA DIAGNOSIS

1. Microscopy

Thick and thin blood smear study is the Gold Standard method for malaria diagnosis.

The procedure involves:

1. collection of peripheral blood,
2. Preparation of thick and thin smear staining of smear with Giemsa stain andexamination of red blood cells for malaria parasites under the microscope.

In our study Giemsa stain was used in Thick smear and Leishman stain used in thin smear study Both of them come under rowmanowsky stain.

Thick smear.

- Thick smears are mainly used to detect infection and to estimate parasitemia.
- Giemsa stain is recommended by WHO for thick smear staining.

- Quality of Staining.

A properly stained blood film is critical for malaria diagnosis, especially for precise identification of malaria species.

Use of Giemsa stain is the recommended and most reliable procedure for staining thick and thin blood films.

Giemsa solution is composed of eosin and methylene blue (azure).The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue. The thin film is fixed with methanol.

De-haemoglobinization of the thick film and staining take place at the same time. The ideal pH for demonstrating stippling of the parasites to allow proper species identification is 7.2.

The two methods for staining with Giemsa stain are the rapid (10% stain working solution) and the slow (3% stain working solution) methods.

The rapid (10% stain working solution) methodit is most more efficient stain help in quick diagnosis compared to slow(3% stain working solution). We used 10% stain working in our study for thick smear. [26]

Thin smear.

Thin smears allow the examiner to identify malaria species, quantify parasitemia, and recognize parasite forms like schizonts and gametocytes.The

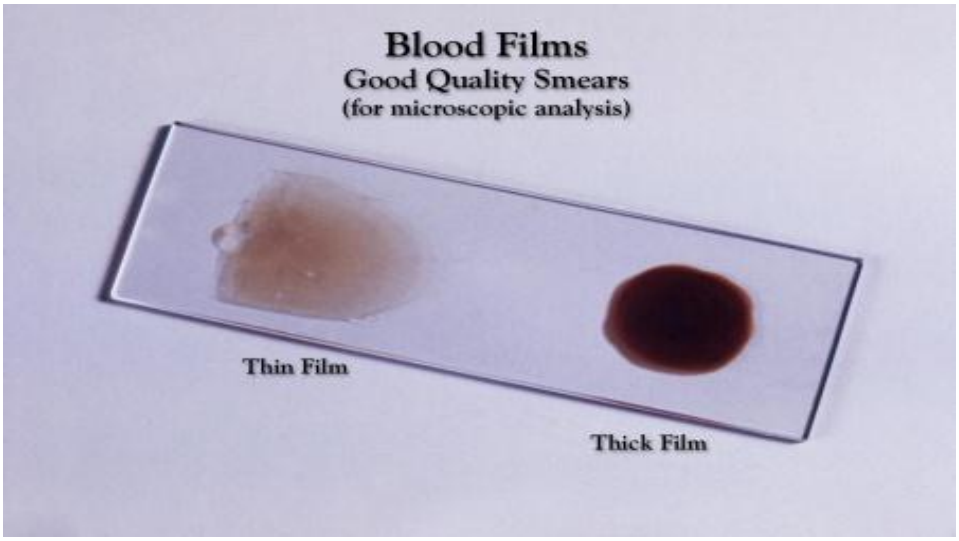
diagnostic accuracy depends on quality of blood smear ,skill of pathologist and parasite density levels.

PIT FALLS IN THIN SMEAR

- False positive- Artifacts and large platelet and poor quality smear can misdiagnose a malaria infection.
- False negative. Happens when the there is low mild parasitemia as well as well as when examiner see less than 100 oil fields before declaring asmear negative which is not possible in our study as strict WHO protocol is followed in reporting a malaria suspect smear and slides are reviewed by more than one observer.

Very low level parasitemia can miss can miss the eye of even experienced reporter.

FIG 6 : THICK AND THIN BLOOD SMEAR (Source: CDC)



LEVEL OF PARASITEMIA IN MALARIAL INFECTION

Level of parasitemia is important to identify the severity of malarial infection.In Plasmodium falciparum malaria which is severe among the various malarial infection has high parasitemia when compared to plasmodium vivax which usually causes mild to moderate parasitemia and very rarely severe parasitemia.

Unlike vivax true parasitemia is difficult to find in falciparum because in vivax all the asexual erythrocytic schizogony seen in peripheral blood film but in malaria all the stages are not found periperal blood film where ring forms are common.

Also many parasites will be seeding the internal organ and circulation making difficult to find the actual parasitic load in falciparum malaria.Again the level of parasitemia is also mild to moderate unlike falciparum causing complicated malaria which is lethal to the person infected.

- Plasmodium ovale and malariae not reported in our study.
- Thick smear and thin smear both can be used for calculating parasitemia.
- Thick smear is more reliable. Thin smear is used when parasites in each thick film is more than or 100 as usually seen falciparum malaria to calculate the number of parasite infected.

PROCEDURE FOR PARASITE COUNTINGTHICK SMEAR AND THIN SMEAR

One has to look for field with white blood cells are Seen start to count the parasite from that field in athick smear.

Depending on the number of parasites observed , stop counting after you have examined 200 or 500 WBC.

If Number of parasites counted is greater than or equal 100 counted in 200 WBC stop counting and record the total parasites counted.

If the number of parasites is less than or equal to 99 parasite then count 500 WBC stop counting and record the results as the number of parasites per 500 WBC.

As suggested by WHO calculate the parasite density counted using average white cell count of 8000/µL using Formula[27].

$$\text{Parasites / µL} = \frac{\text{Number of parasites counted}}{\text{Blood}} \times 8000 \text{ white blood cells/µL}$$

No. of white blood cells counted(200wbc)

If more than equal to 100 parasites seen in each thick film than perform the thin smear and count the parasitic density as per the formula.

Parasites/Microlitre= {Number of parasitized red blood cells x5000000 } divided by number of RBC countedjust like in thick smear only asexual forms are counted. gametes are documented but are not counted.

Stop counting when about 20 fields with about 250 RBC per highpower field(about 5000 rbc) have been counted.

The malaria parasites were examined and the approximate numbers of parasites were recorded as per WHO guidelines but graded as per alternate method .

In our study we used + signs to Grade the severity of parasitemia (Alternate method) by fitting in the Parasitic count as per WHO calculation.

1+- mild parasitemia	between 0-40 Parasites /micro litre
2+ - mild parasitemia	between 41-400 parasites /micro litre
3+-Moderate parasitemia	between 401 to 4000 parasites / microlitre
4+ -Severe parasitemia	more than 4000 parasites/ microlitre

Most of the cases in our study were in moderate parasitemia and few cases were mild parasitemia. One case of very severe parasitemia was reported with smear positive for falciparum malaria

b) Quantative Buffy Coat (QBC) test

This method involves centrifuged and compressed red blood cell layer stained with acridine orange and then examinated under an ultra-violet light source.

The whole procedure takes place in a glass hematocrit tube which is precoated internally with acridine orange stain and potassium oxalate; it is filled with 55-65 μl of blood.

The tube is centrifuged and so the components separate according to their densities forming bands

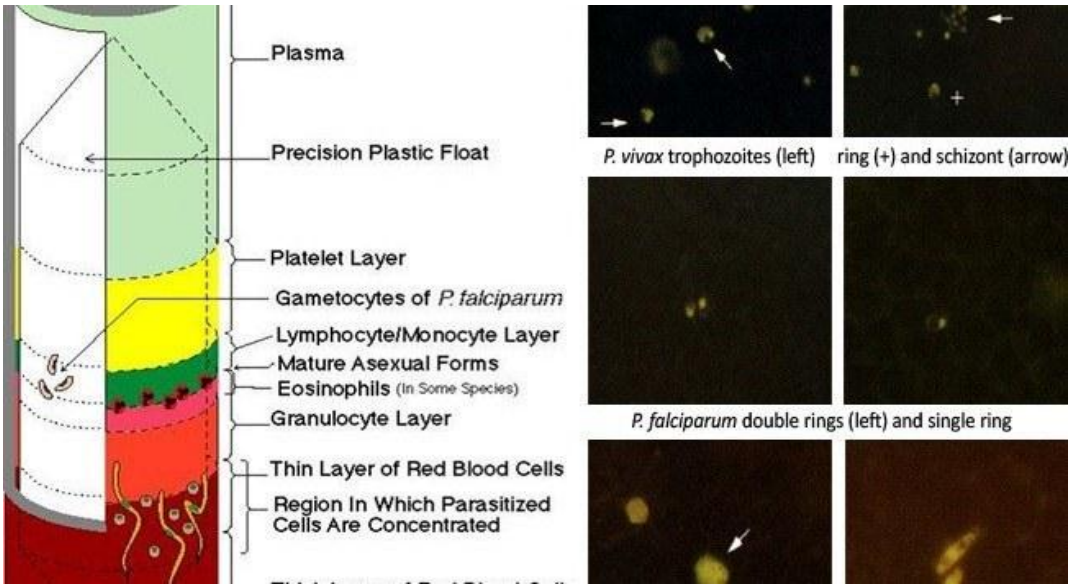
Fluorescing parasites are then observed, with a UV microscope, at the red blood cell/white blood cell interface as shown bellow in Figure 4.

QBC test is easier and faster than classic peripheral blood smear microscopy but the equipment required is expensive and species identification and accurate enumeration are impossible.

QBC Malaria Test is 5.5 to 7% more sensitive than Giemsa thick films. It can detect as little as 1 parasite per μL of blood and establish diagnosis earlier than thick film in 47% of low parasitemia (<10 parasites per μL) cases.

In our study we used QBC to double confirm the diagnosis of malaria in all cases.

FIG 7 [28]



Antigen-based techniques

Rapid Diagnostic Test (RDT)

RDT is a device that can detect malaria antigen in a small amount of blood (5µl) by immunochromatographic assay (colour change in an absorbing nitrocellulose strip) with monoclonal antibodies directed against the parasite antigen. Depending on the target antigen, rapid tests that now exist may involve combinations of the following:

- HRP-2 (Histidine Rich Protein-2) is a protein produced by the asexual stages and gametocytes of *P. falciparum*, expressed on the membrane of red blood cells (sensitivity: detects parasitemia of >40 parasites/ µl). It often persists in patient's blood for weeks after successful treatment.
- Plasmodium aldolase is an enzyme of the parasite glycolytic pathway expressed by all malaria species (pan malarial antigen- PMA).
- Lactate dehydrogonase (LDH) is a glycolytic enzyme produced by asexual and sexual stages of parasites and released by infected red blood cells. (sensitivity: detects parasitemia of >100 parasites/ µl)

Advantages:

- Simple to perform and to interpret with a result given in 15- 30 minutes
- Ability for outbreak investigation and screening febrile returnees from endemic areas
- HRP-2 tests have sensitivity of> 90% for *P. falciparum* and LDH tests >95%

Disadvantages:

- Low sensitivity in detecting asymptomatic patients particularly in low parasitemias
- Cross reactions to autoantibodies (such as rheumatoid factor in case of HRP2 test)
- False positivity (gametocytemia, persistent asexual-stage parasitemia below microscopy detection limit)
- False negativity (genetic deletion or mutation of hrp-2 antigen)

Hence Anti Body based Based RDT banned in india and Antigen Based RDT is still used due to good sensitivity ty and specificity as an alternate method in field survey and mass screening till date.

Though confirmation of Positive cases with microscopy is warranted to avoid wrong diagnosis which is still possible with RDT .

3. Molecular techniques

Polymerase Chain Reaction (PCR)

Using PCR amplification, it is possible to detect all 4 species of malaria parasites with a reportedly 10-fold greater sensitivity than microscopy.

Useful in detecting plasmodium infections in healthy adults with "submicroscopic" levels of parasitemia.

Advantages:

- High sensitivity and specificity
- Quantitative determination of parasite and species differentiation ability

Disadvantages:

- Unsuitable for field conditions
- Expensive
- Technically demanding

Hematological abnormalities are considered a hallmark of malaria various studies have confirmed the same.

In the Era of Automated analysers all the hematological parameters are generated in few minutes with the new technologies updated from time to time resulting in decreased turn over time in generating results.

These new generation automated analysers are more sensitive and accurate in generating unbiased reports if proper quality control is maintained

These automated analysers can prove handy in recording various hematological parameters and histogram flagging in a patient with malaria.

Hence such a machine generated reports and histogram flags can be used at par with microscopy which is gold standard test for diagnosing malaria at the primary health care level in rural and semi urban areas by semi skilled heath

workers where access to modern health care is still lacking and where there is huge load of malaria cases at the community level.

Most common complaints was fever with chills which makes the clinician suspicious for malaria in endemic zone.

Most Common hematological parameters affected in malarial infection includeAnemia,Thrombocytopenia,Leucopenia or leucocytosis, lymphopenia, monocytosis and pseudo eosinophilia has been documented in various studies.

Mechanism involved in anemia

Anaemia is common clinical manifesation in plasmodium vivax and falciparum which is supported by lab values of Haemoglobin , hematocrit and RBC count.

Plasmodium vivax has a very strong predilection for young red blood cells in particular reticulocytes.

P. falciparum significant infect older cells though young RBC can also be involved. [29-31]

Erythrocytes infected by either species is to host the replicating parasite for approximately 48 hours before bursting and releasing daughter merozoites.

The range of peripheral parasitemia in P. vivax infections is lower in vivax usually not exceeding 2% it is high in plasmodium falciparum malaria even go upto 10%[32].

Premature death of infected reticulocytes due to P. vivax infection is the cause of anemia due to reduced production of mature RBC in the population infected with Plasmodium vivax [32-34].

In P. falciparum malaria, the mechanism is different since it infects mature RBC than young RBC that is reticulocytes.

Possible mechanisms

Impaired erythropoiesis,removal of both infected and non infected RBC from the circulation [35-38].

Parasitaemia is typically lower in vivax compared with falciparum infections, absolute number of red blood cells removed from circulation is more in vivax, and hence the degree of anaemia resulting from infection by the two species, is often similar [39-41]

In P. vivax malaria, approximately 34 non-infected cells are cleared for every one infected cell [42]whereas in P. falciparum malaria, this ratio is closer to 8 to 1 [43][44].

As in falciparum malaria, vivax-infected erythrocytes adhere to uninfected red blood cells (rosetting) [45]but unlike falciparum-infected cells, they have limited propensity to adhere to endothelial cells and, therefore, sequestration in the deep microvasculature is not a major factor in the pathogenesis of vivax malaria [46][47] which makes vivax infection less severe.

Erythrocytes parasitized by P. falciparum become less deformable than uninfected red cells and have reduced capacity to pass through narrow inter-endothelial slits in the wall of splenic sinuses (mean dimensions $1.89 \times 0.65\ \mu m$.[48-51]

Vivax-infected cells become more deformable as the parasite matures and are thought to retain the ability to squeeze through splenic slits [48,52,53]

In falciparum malaria, red blood cell sequestration reduces the proportion of parasitized red blood cells that traverse the spleen.

Increased deformability of infected red blood cells in vivax malaria may limit the proportion of red cells that are removed during passage through the splenic microcirculation.

P. falciparum and P. vivax have evolved two different means of escaping splenic filtration.

In both vivax and falciparum malaria, parasitized, and possibly non-parasitized, red cells are hypothesized to be more fragile than red cells in non-infected individuals and more prone to damage from shear stresses[48,52,54-55]. This process is potentially a more important cause of red cell loss in falciparum malaria since in this disease, major sequestration in the microvasculature impedes the passage of circulating erythrocytes and erythrocyte rosettes [56] which causes intra vascular hemolysis.

In addition to these mechanical processes, activation of the innate, cell-mediated and humoral immune systems in response to the presence of P. vivax antigens enhances the detection and removal of infected and abnormal uninfected red blood cells [57-59].

The non-specific immune response for a given parasitaemia is greater for P. vivax than P. falciparum and may partially explain the greater proportional removal of non-parasitized cells and lower fever threshold in vivax malaria[60-62].

- Reduced glutathione, which is necessary for protecting red cells against damaging oxygen species, is reported to be depleted in vivax malaria [63]
- Infection withP. falciparum causes altered expression of complement components and deposition of parasite proteins on infected and uninfected red blood cells [64] facilitating opsonization and complement-mediated phagocytosis [65-66]It is unknown whether these processes also occur in vivax anaemia**.**

The role of the spleen in vivax malaria is poorly understood though splenic enlargement in this infection appears to be similar to falciparum malaria.[67-68] Indeed vivax malaria carries a very low but well-known risk of splenic rupture; considered greater than for falciparum malaria [69-70]

Increased removal and destruction of both infected and uninfected red cells in vivax malaria is most prominent during the early stages of infection however

enhanced removal of uninfected cells persists for five weeks or more after effective treatment of blood-stage infection [71-72]

In chronic, asymptomatic vivax parasitaemia, common in vivax-endemic areas, removal of both infected and uninfected red cells is likely to persist for the duration of infection.

With the above mechanisms explained its obvious that one should expect anaemia in vivax and faciparum in the CBC parameters recorded by the automated analysers.

In our study except for the single case of plasmodium falciparum malaria (n=1)all other are caused by plasmodium vivax(n=59)

Anaemia was present in 51 out of 60 cases and had asignificant p value of 0.002 .

Again in anaemia significant p value of 0.024 was seen when compared with degree of parasitemia.Anaemia was propotional to the degree of parasitemia.

Mechanism involved in thrombocytopenia

A) parasite killing B) Erythrocyte sequestration C) inflammation

Platelets have major role in the pathogenesis of malaria .Possible mechanisms of platelet involvement in malaria.

Platelets bind to infected erythrocytes release of PF4 which is lethal to parasitised RBC [73][74]

Platelets tethered to ultralarge VWF strings secreted from activated ECs can promote cytoadhesion and sequestration of IEs, thereby promoting vascular occlusion [75]

Platelets have also been reported to be important in enhancing IE clumping, which further contributes to microvasculature occlusion in malaria.[76]

Platelets also play an important role in driving development of [77]Inflammation and EC activation, both of which are critical in malaria pathogenesis.

Platelets can impact malaria pathogenesis, which is multifactorial,it is perhaps not surprising that previous studies have produced conflicting results about the importance of platelet-mediated effects.[78]

Kho et al provide further evidence that platelets play a major role in the pathogenesis of malaria infection.[79]In particular, they demonstrate that platelets can kill circulating parasites of all major Plasmodium species in human malaria

Based upon their findings, the authors estimated that overall, platelets may kill up to 20% of circulating blood-stage Plasmodium in clinical malaria.

In cases of P vivax infection, this platelet-killing phenomenon may actually account for as many as 60% of the total intraerythrocytic parasitesstudy by

Chandra et al., an increased MPV was observed in malaria patients in which the pathogenesis of thrombocytopenia was due to increased peripheral destruction (hyperdestuctive) while decreased MPV was attributed to bone marrow disease as a result of hypoproductive state [6]. An increase in MPV was higher in vivax infected 19 cases (28.78%) in the present series.

Panasiuk et al., and Conte et al., found that Platelet-Associated IgG (PAIgG) is increased in malaria and is associated with thrombocytopenia .

Previous studies have reported that extent of thrombocytopenia correlates with parasite density, severity of malaria infection, and clinical outcomes.

Together, these data support the hypothesis that platelets are important in malaria pathogenesis.

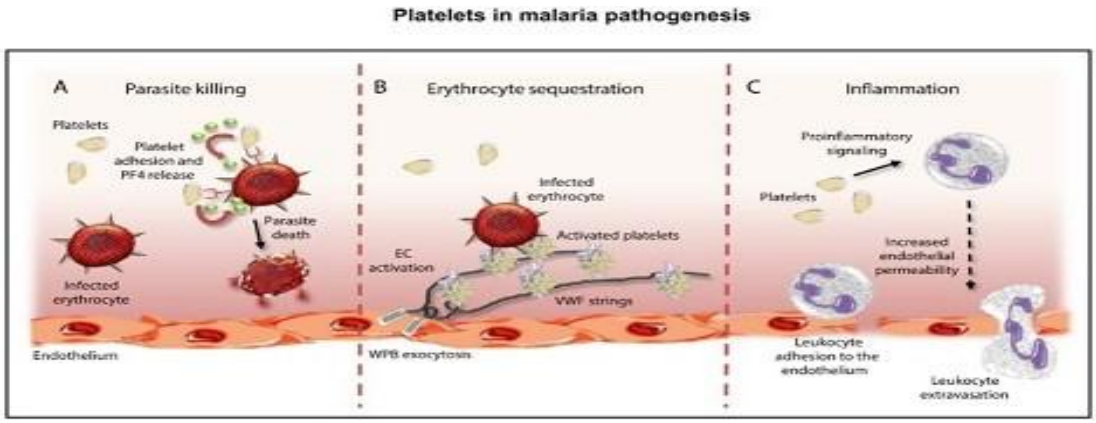
In our study Thrombocytopenia emerged as a strong predictor of malarial infection. in clinical suspeced cases of malarial fever as well as in smear positive malaria cases

The degree of parasitemia was directly propotional to the degree of thrombocytopenia. which means severe parasitemia will have severe thrombocytopenia.

Apart from thrombocyytopenia, PCT is also lowered in malaria positive cases .

Among the malaria positive smears MPV appears elevated along with P CLR was elevated in malaria positive cases indirectly pointing out the presence of giant platelet in a setting of peripheral destruction of platelets as suggested in the mechanism of thrombocytopenia in malaria.

FIG.7 PLATELET IN MALARIA PATHOGENESIS



Jamie M. O'Sullivan,James S. O'Donnell, Platelets in malaria pathogenesis, Blood, 2018,

https://ashpublications.org/blood/article/132/12/1222/39621/Platelets-in-malaria-pathogenesis[79]

Monocytosis in malaria

The most recent advances prove that monocytes are a key component to control parasite burden and to protect host from disease.

Monocytes' protective roles include phagocytosis, cytokine production and antigen presentation. [80]

Monocytes can be involved in pathogenesis and drive inflammation and sequestration of infected red blood cells in organs such as the brain, placenta or

lungs by secreting cytokines that upregulate expression of endothelial adhesion receptors.

Monoctytes engulf the hemozoin pigment like the neutrophil which come as the first line defence in malarial infection. Plasmodium DNA, hemozoin or extracellular vesicles can impair the function of monocytes. With time, reinfections with Plasmodium change the relative proportion of monocyte subsets and their physical properties. These changes relate to clinical outcomes and might constitute informative biomarkers of immunity.

Monocytes engulf the hemozoin pigment like the neutrophil which come as the first line defence in malarial infection. Rise of monocytosis is common in malarial fever but in our study we could not get positive p value for the sample examined. Though there was apparent increase in the malaria positive samples though not statistically significant which could be due viral infections like dengue etc which too can cause monocytosis.

SPURIOUS EOSINOPHILIA/PSEUDOEOSINOPHILIA

The Cell-Dyn (CD) 3500 (Abbott Diagnostics) is the first automated analyzer to detect malaria hemozoin in monocytes according to their abnormal depolarizing pattern. [81-82]

Hemozoin-containing neutrophils are probably misidentified as eosinophil, because other features of neutrophils are shared with eosinophils. [83]

Hemozoin is produced by maturing malarial parasites and is the end product of hemoglobin digestion, consisting of a polymer of heme groups.[84-85]

Our study shows significant p value <0.001 among malaria positive cases and severe parasitemia is associated with greater spurious eosinophil count excluding one case of true eosinophilia with high percentage .

Pseudoeosinophila which is common in malarial infection which is manifesed by spurious increase of eosino phil count. The FLAG in mixed population of F2 region in WBC histogram in 3 part analyser one should suspect pseudo eosinophilia or monocytosis. These pseudo eosinophils are none other than the hemozoin ingested neutrophils. Out of 60 smear positive cases 20 had elevated count above cut off in eosinophil count.

On manual examination 19 of them showed normal eosinophil count.One sample had eosinophil percentage of 35 % confirmed by microscopy. The results comparing severity of parasitemia shows severe parasitemia has more eosinophil count than moderate level

Due to one case among 5 cases of mild parasitemia showing true eosinophilia in excess of 35% because of which p value for pseudoeosinophilia was not highly significant among the malaria positive samples based on severity of eosinophilia.

Hence eosinophilia in malaria suspect should be reviewed for pseudo eosinophilia and mixed population flag in wbc histogram.

True eosinophilia has to be ruled out because in any parasitic infection true eosinophil count too can be elevated just as we saw one case with true eosinophilia in our study.

Sensitivity of pseudo eosinophilia (smear positive)in our study is 19/60 x 100 =31.66%

Specificity of pseudo eosinophilia (smear negative)in our study is 135/140x100=96.42%

Several authors have described pseudoeosinophilia or abnormal WBC scattergrams as a result of hemozoin-containing neutrophils using the Sysmex XE-2100 analyzer. [86-88]

Hemozoin in WBC seems to be a sensitive indicator of prognosis, an automated hematology analyzer may offer a new way to assess disease severity.[89]

In this study, the overall sensitivity (46.2%) and specificity (99.7%) of the Sysmex XE-2100 analyzer for the detection of malaria were lower than in the previous study of South Korea (69.4% and 100%, respectively). [87]

In several studies, the percentage of hemozoin-containing WBCs varied according to populations, age, and disease activity. 13–15 A remarkable reduction in sensitivity in this study was probably caused by the presence of younger and

fewer circulating parasites, severity of infection, and host immunity factors, 16–18 which produced less hemozoin and were below the analyzer detection limits.

Our study showed sensitivity of 31.66 % and high specificity of 96.42% for psedoeosinophilia.

The low sensitivity can be less severe infection in the current study involving only eight cases out of 60 smear positive cases causing severe parasitemia which accounts for just 13.3 % of total malaria positive cases.
Our study shows moderate to severe thrombocytopenia in in 56 smear positive cases.(93.3%).

Hence pseudo eosinophilia was normally anticipated. since the number of severe parasitemia is less causing low sensitivity is seen in our study.

LEUCOPENIA IN MALARIA

WBC counts are generally lower in malaria patients compared to healthy patients and that there is a trend towards lower WBC counts in patients infected with either P. falciparum or P. vivax [90].

These changes are dynamic during illness, with lower lymphocyte and eosinophil counts at baseline and higher counts as the symptoms disappear [90-91]leukocytosis, shows presence of coinfections, and gram negative bacteremia in complicated cases of P. falciparum malaria [92].

Several theories attempt to explain these described hematologic disorders,

i) bone marrow (BM) suppression secondary to immune response imbalances,

ii) lower average cell life, and

iii) leukocyte redistribution to lymphoid organs and tissues with increased inflammatory response or sequestration in microvasculature [93-97]

The leukogram profile was similar in P. vivax and P. falciparum malaria. Although a strong inflammatory response in P. vivax infections has been recognized.[98]Our study did not find a differential leukocyte response by Plasmodium species, except for patients with severe thrombocytopenia and P. vivax infection, whose leukocyte counts were significantly lower, in accordance with previous findings in the acute phase of infection.Leukopenia is a common finding in falciparum and vivax malaria [90-91, 99].

Some studies indicate that there is a greater trend towards leukopenia in infections with P. falciparum, this also happens with P. vivax [90,100,101].Therefore the presence of leukopenia or its severity does not seem to be useful for diagnosis of species.Leukocytosis frequency in malaria varies from one study to another [93,102] and is reported predominantly in falciparum malaria.

In falciparum malaria, leukocytosis has been explained by

1) the stimulus of infection on the BM to release leukocytes during the paroxysms[90];
2) increased proinflammatory cytokines could favor the exit of leukocytes from BM [94].
3) by the presence of bacterial or viral coinfections[103], considering that these possible diagnoses were not excluded in some of the studies [91].

Overall Leucopenia is common in malaria our study showed significant p value for leucopenia as well as the degree of parasitemia was propotional to the parasitic density.

LYMPHOPENIA IN MALARIA

Lymphopenia is a well-established feature of P. falciparum malaria but is replaced by lymphocytosis in a matter of a few days after initiation of drug therapy, before gradually normalizing over the next couple of weeks [105].

Initial lymphopenia reflects disease-induced reallocation of T cells to sites of inflammation[2,104], followed by reemergence of such cells once cured [105].Kern et al.(106) and Matsumoto et al.(107) provide evidence of both lymphopenia and increases in soluble Fas ligand, both fail to demonstrate any evidence that one is the consequence of the other.

Others have pointed to the increased propensity of lymphocytes from malaria patients to undergo spontaneous apoptosis in vitro possibly induced by soluble Fas ligand (sFasL)-Fas interaction .

Fas-induced apoptosis thus appears to be involved in T lymphopenia during malaria infection.T lymphopenia appears to play a crucial role in the pathogenesis of malaria but is not yet well characterized.Lymphocytopenia has frequently been described in malaria patients in endemic areas and was found to be present in 63% of patients with imported Plasmodium falciparum infection.

Studies on the correlation between lymphocyte count and malaria severity yielded conflicting results, as both lymphocytopenia and lymphocytosis have been reported to be associated with adverse outcome.In our study showed significant p value for lymphopenia but was not relevent when compared to the degree of parasitemia.

In our study showed significant p value for lymphopenia but was not relevant when compared to the degree of parasitemia.Hematology analyzers are used to count and identify blood cells at high speed and accuracy.The early hematology analyzers relied on only Coulter's Principle.New generation automated analyzers detect various parameter in a short time more accurately .

COULTER PRINCIPLE

Named after Wallace Coulter, the Coulter principle states that particles passing through an orifice (along with an electrical current) will produce an increase in impedance, due to the displacement of electrolytes caused by the presence of the particle.

This change in impedance is proportional to the volume of the particle. The Coulter principle has been used for particle counting and sizing in a variety of fields.

Its use in field of hematology has been revolutionised by the invention of automated analysers which made Hematology look simple with the new evolving new generation automated analysers which can read so many parameters in a short time.

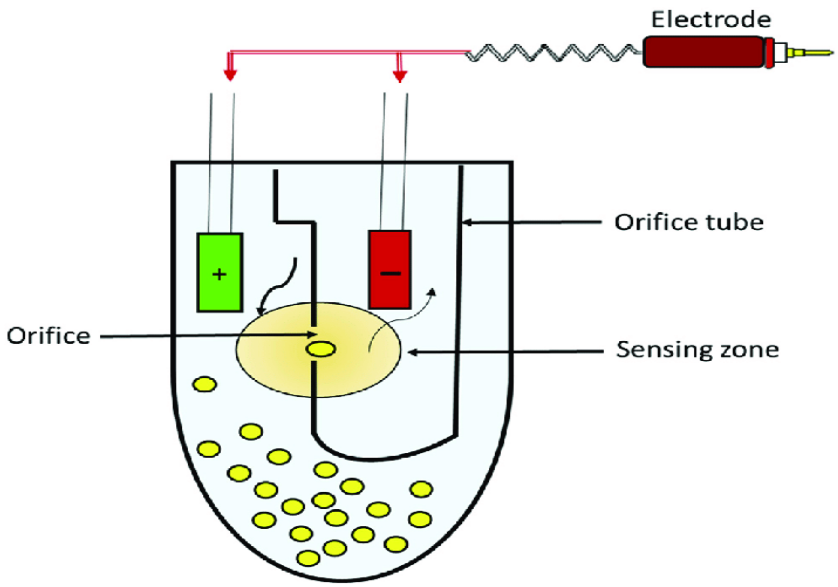


FIG.8 COULTER PRINCIPLE

Source:https://www.researchgate.net/figure/Principle-of-Coulter_fig3_326819577 [108]

In 3 part Differential cell counter

Testing method is bioelectrical impedence techique.It combine with signal generator , amplifier, recognizer threshold adjustor,coulter system and automatic compensator.

In 5-Part Differential Cell Counter

This type of hematology analyzer utilizes both Coulter's Principle and flow cytometry to determine the granularity, diameter, and inner complexity of the cells.

Using hydrodynamic focusing, the cells are sent through an aperture one cell at a time. During this, a laser is directed at them, and the scattered light is measured at multiple angles. The absorbance is also recorded.The cell can be identified based on the intensity of the scattered light and the level of absorbance.

A 5-part cell counter can differentiate all WBC types (neutrophils, lymphocytes, basophils, eosinophils, and monocytes).

Hematology analyzers are used to conduct a complete blood count (CBC), which include ;

1) red blood cell (RBC) and its other parameters like hemoglobin, Haematocrit , RBC distribution width RDW ,Mean corpuscular volume MCV,Mean corpuscular hemoglobin MCH,Mean corpuscular hemoglobin concentrations MCHC.

2) white blood cell (WBC) and its parameters like WBC differential count in percentage and absolute value

3) Platelet count and its parameters like

- Mean Platelet volume
- Platelet distribution width
- Plateletcrit
- Platelet mean volume
- Large platelet cell ratio

FIG 9 Sysmax XN1000 (5 PART) AND SYSMAX XP 100(3 PART) which isalso used in our study.

SYSMAX XN1000

SYSMAX XP100

In 3 part automated analysers there is Pre-differentiation of the white blood cells into three subpopulations. The major advantage of automated cell differentiation is speed and enhanced precision and accuracy.

Sysmex distinguishes lymphocytes, neutrophils and a mixed population consisting of monocytes, basophils and eosinophils.

This mixed population composed of monocytes and eosinophils which is very important flag in histogram in our study since 5 part analyser used in our study donot generate histogram instead they generate scattergram which is not a part of our study.[109]

Both 5 part and 3 part analysers are used in our study from the same manufacturer SYSMAX.The total samples of malarial fever suspect cases are run both in 3 part as well as part and documented.Each machine it self has a inbuilt internal quality check apart from it .

Daily QC is done every day among different 3 part machines and between 5 part and 3 part machine to standardise the possible error there by making sure that error reports are avoided.

CBC AND HISTOGRAMS ARE GENERATED

Histogram composed of RBC, platelet histogram is generated in 5 part analyser while wbc scattergram is generated in 5 part analyser scattergram abnormalities are not documented in the current study.

WBC Histogram is not generated in 5 part analyser hence the sample is run on 3 part analyser to document the WBC histogram flags.

TABLE 4.CBC CUT OFF VALUE AS PER WHO

Hematological variables	WHO CRITERIA
RBC count (in million)	<4.2 million/cmm- Male <3.6million/cmm-Female
Haemoglobin (Hb)g/dl	<13g/dl -Male <12g/dl-Female
Haematocrit (Hct)%	<41%-male <36%-female
Mean Corpuscular volume(MCV) fl	<80fl
Mean Corpuscular Haemoglobin (MCH) pg	<27pg
Mean Corpuscular Haemoglobin concentration MCHC g/dl	<32g/dl
Total Leucocyte count TLC (thousands)	<4000/cmm
Platelet count	<1.5Lakh/cmm

We have taken the WHO criteria for our study.

Hematological variables were divided into three parts as RBC , WBC and PLATLET Indices and Their Histogram finding and Flags are documented in Fever suspected for Malarial Infection which is then compared for any significant finding and correlation is derived .

Hematocrit

Measures the amount of RBC in the blood

Increases when RBC number increases or plasma level drops

Decreases due to RBC production decrease , blood loss or RBC Destruction.

RBC INDICES INCLUDE

- Mean cell (or corpuscular) volume (MCV)
- Mean cell hemoglobin (MCH)
- Mean cell hemoglobin concentration (MCHC)

MCV

MCV refers to the average size of the RBCs constituting the sample. Should a mixture of cell populations be present, the sizes of the red cells will be averaged. Reporting units is femtoliters (fL). One femtoliter is 10^{-15} L. Reference interval for adults is typically 80 - 96fL.

- < 80 Fl seen in microcytic anaemia
- >96Fl seen in macrocytic anaemia

MCH

Mean cell hemoglobin (MCH) refers to the average weight of hemoglobin in the RBCs in the sample. Should a mixture of cell populations be present, the weights of the evaluated cells will be averaged. Reporting units is picograms (pg). One picogram is 10^{-12} grams. The reference interval for adults is typically 26-32pg.

MCHC

Mean cell hemoglobin concentration (MCHC) refers to the average concentration of hemoglobin in the RBCs contained within the sample. Should a mixture of cell populations be present, the hemoglobin concentration within the

evaluated cells will be averaged. Reporting units is g/dL. Reference interval for adults is typically 32 - 36 g/dL.

HISTOGRAM FLAGS

RBC HISTOGRAM FLAGS

FIG 10 .NORMAL RBC HISTOGRAM

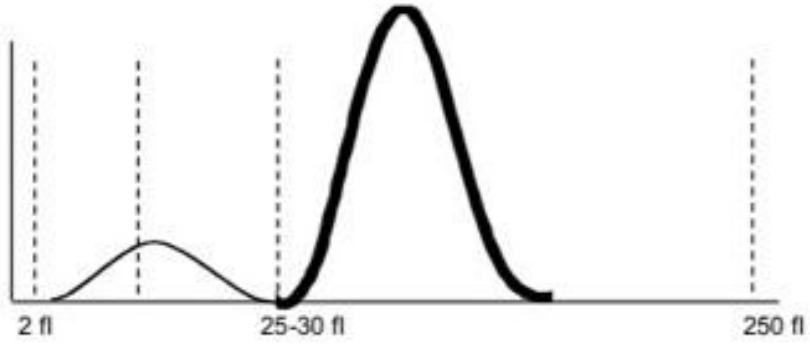
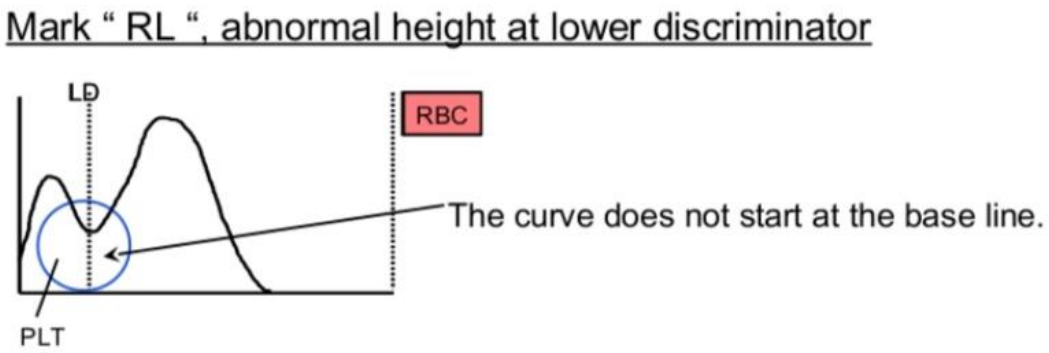


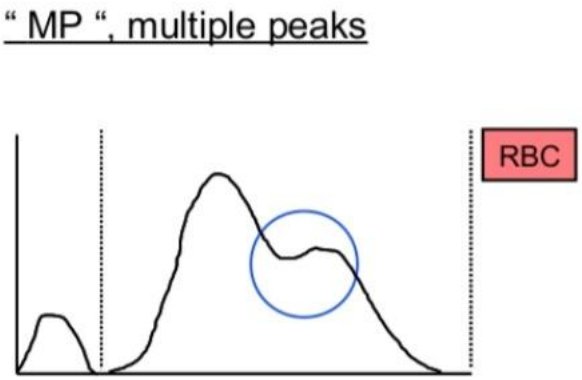
FIG 11.RL FLAG IN RBC HISTOGRAM [110]



POSSIBLE CAUSES

1. Giant platelets
2. Micro Erythrocytes
3. platelet clumps.

FIG . 12 .MULTIPLE PEAKS(MP) FLAG IN RBC HISTOGRAM

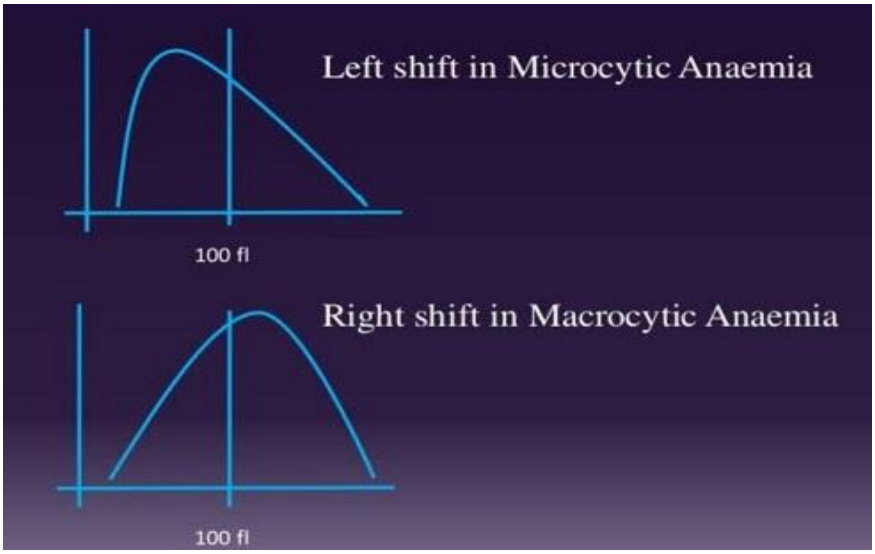


POSSIBLE CAUSES

Iron deficiency Anaemia

Post Blood transfusion(own RBC and donor RBC)

FIG 13 : RBC HISTOGRAM FOR MICROCYTIC AND MACROCYTIC ANEMIA[112]



Normal RDW - CV is 11-16%

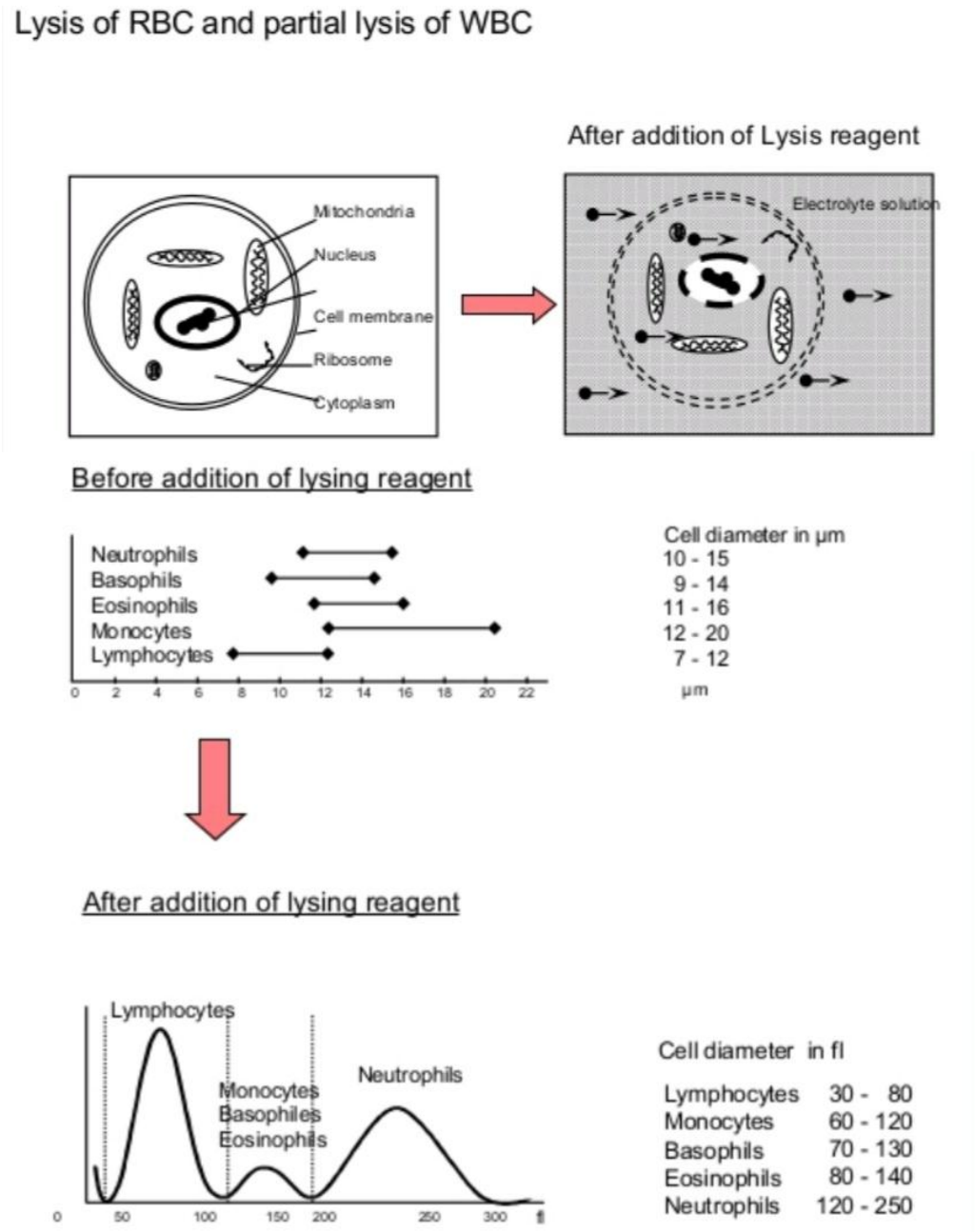
RDW - SD normal value is 37-46 fl.

RDW-CV which is considered sensitive for anisocytosis was significant in our current study with significant p value among malaria positive cases when compared with non malarial fever as control.

WBC HISTOGRAM AND FLAGGINGS

FIG.14

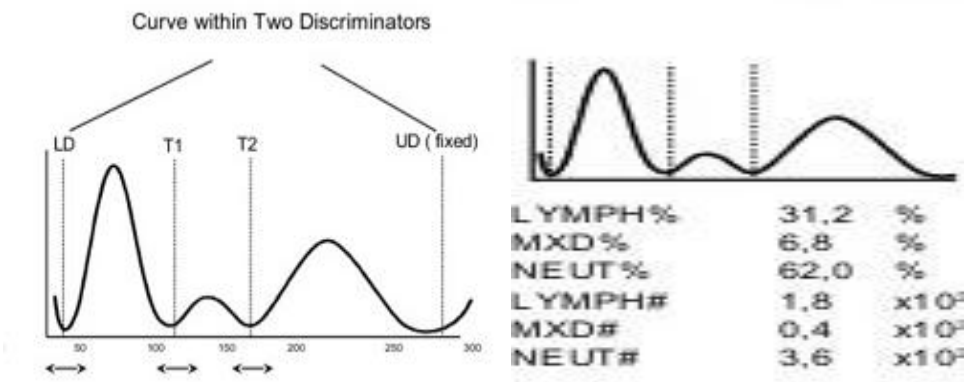
Principle behind generation of WBC curves in histogram



T1 And T 2 are valley discriminators defined by plateau.

These discriminators seperate the leucocyte population into 3 population namelyF1 - Lymphocytes F-2- Mixed F-3 Neutrophils.

FIG 15.NORMAL WBC HISTOGRAM (3 PART ANALYZER)



In our study the mixed population is increased in 57 out of 60 cases studied which account for true positive cases

The rest 3 cases where histogram appeared normal but was positive in Peripheral smear constituted True Negative cases . Mean while 113 cases among smear negatives cases showed no deflections in the mixed population.which are True negatives.

The 27 smear negative cases showing positive peak in the mixed population is False positive case.

FIG 15 F1 F2 F3 REGION IN HISTOGRAM

3. Flag "F1" , "F2" and "F3"

The Histogram of the Leukocytes is located within the external discriminators LD and UD.

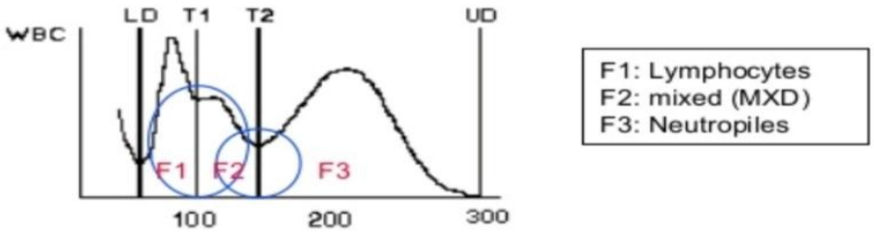
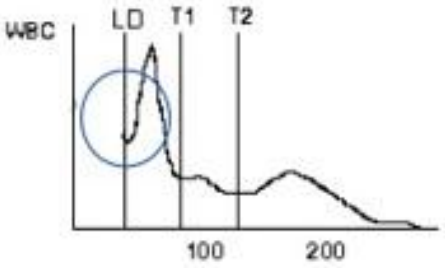


FIG 16 .WL FLAG IN WBC HISTOGRAM

Flag " WL ", curve does not start at the base line



Causes

1) Platelet clumps which can be due to EDTA incompatibility and clotted sample.
2) Highly osmotic resistance (Erythrocytes not lysed) Which is highly sensitive finding in parasitised RBC in malarial infection .Hence a flag in this region one should should also keep malaria in the differential diagnosis.
3) Erythroblast(NRBC)

Which can give a false flag is ruled out in current study. since they are counted both manually and by 5 part analyser

Shamsi TS et al [120]enumerates the causes of WL flag as the presence of nucleated red blood cells, giant platelet, small platelet clumps, moderate number of target cells or polychromatophilic RBCs and malarial parasites.

Platelet Histogram and flaggings.

A normal platelet count ranges from 150,000 to 450,000 platelets per microliter of blood. Having more than 450,000 platelets is a condition called thrombocytosis; having less than 150,000 is known as thrombocytopenia.

Severity of thrombocytopenia

- Mild less than 1.5 lakh to 1 lakh
- Moderate 1 lakh to 50000
- Severe less than 50000
- Very severe clinically significant - less than 20000

In our study moderate to severe thrombocytopenia was common.Clinically severe requiring blood transfusion was not documented in the current study which involves more than 98% reported case of plasmodium vivax.

PLATELET INDICES

Platelet indices composed of

1) Platelet count
2) Mean Platelet volume(MPV)
3) Platelet Distribution Width(PDW)
4) Platelet Large Cell Ratio (P-LCR) and Plateletcrit.

Since Thrombocytopenia is strong predictor of anaemia its indices are also significant in interpreting any changes related to malarial infection.

MPV(mean platelet volume)

- Measure of platelet volume expressed in femto litre
- It is derived from dividing plateletcrit by total platelet count similar to MCV. Normal value : 8.6- 11.4 fl

MPV correlates with platelet aggregation .Larger Platelets also express increased levels of adhesion molecule like P - selection, GP, IIb/IIIa Independent of platelet volume. During activation platelets shape change from bi concave disc to spherical and a pronounced pseudopod formation occurs that leads to MPV increase during platelet activation.

MPV affected by anto coagulation, storage temperature and delays in processing. Platelet swelling due to EDTA sample can be minimised by rapid processing of samples with in 1 hour or by using sodium citrate as anti coagulant
In our study MPV was not significant in malaria suspected samples . but showed significant p value in malarias positive samples based on severity of parasitemia. which means severe the parasitemia cause increase in MPV.

PLATELET DISTRIBUTION WIDTH(PDW)

- PDW is indicator of variability in platelet size and is increased in presence of platelet anisocytosis.
- Normal Value:8.3- 25 FL

- PDW was normal in our study population and had no co relation with degree of parasitemia.
- Increased in platelet clumps,microerythrocytes and RBC fragments.
- Which is the cause of platetlet abnormal histogram is common in malaria.

PLATELETCRIT

- (PCT%)=Volume of platelets expressed as percentage of total volume.
- Calculated by formula=Platelet count*MPV/10000
- Normal value : 0.22 - 0.24%
- Plateletcrit is effective tool to screen platelet quantitative abnormalities and is related to platelet count and size.
- PCT% was significant in our study with clinically suspected malaria cases with significant p value of<0.001 and was inversely proportional to the parasitic density. Which mean Severe parasitemia has lowest of PCT %.
- Low PCT is due to thrombocytopenia which obsevered in our study with malarial infection and also it was corelating with the severity of the infection.
- Platelet count and PCT emerged as a strong suspicion for malaria in our study.
- P LCR (%)= Percentage of circulating platelets(>12fl)
- Normal value:15-35%
- It has been used to monitor platelet activity . larger Platelets are more reactivewith more intra cellular granules.
- Increased in platelet clumps,Giant platelets, microerythrocytes.

- PL CR value was significant comparing the severity of parasitemia in malaria positive cases .
- Though no significant value arrived when comparing with malaria negative cases.
- PLCR though non specific can be signicant in malarial infection showing abnormal platelet distribution in the platelet histogram.

FIG 17. NORMAL PLATELET HISTOGRAM

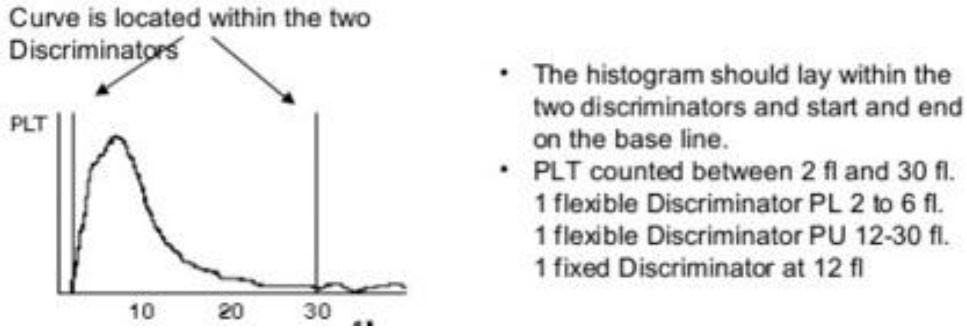
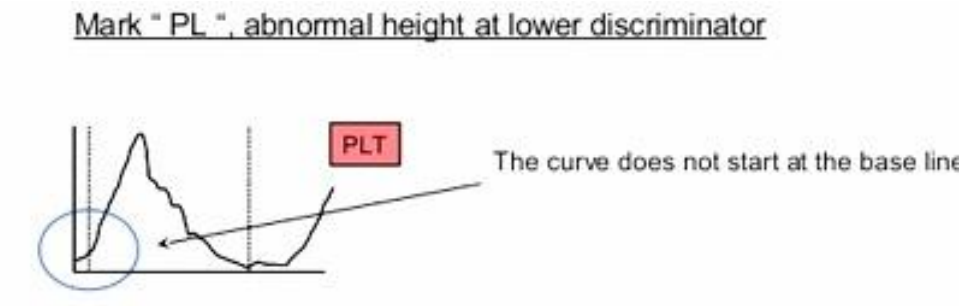
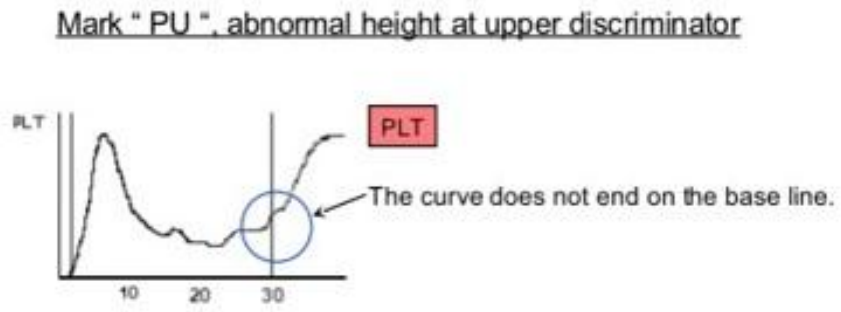


FIG 18. PL FLAG IN PLATELET HISTOGRAM



Possible Causes Cell Fragments

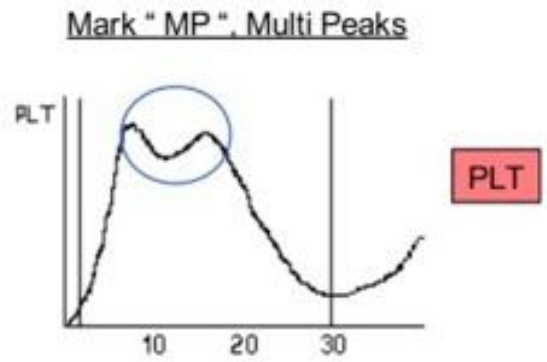
FIG 19 . PU FLAG IN PLATELET HISTOGRAM



Causes

- Platelet clumps
- Giant platelets
- Microerythrocytes
- This flag is important in giving diagnostic clue apart from wbc histogram flags to diagnose malaria.

FIG 20. MP FLAG IN PLATELET HISTOGRAM



Causes

- Platelet transfusion. MP Flag is not seen in our study even though severe thrombocytopenia was reported which was not very severe enough to cause bleeding diathesis and shock like seen in dengue infection.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study Area

The current prospective study is carried out in Rajeev Gandhi Government General Hospital attached to madras medical college chennai.

Study Population

The population of subjects used in this study were 200 individual malaria suspected samples irrespective of age, gender, with clinical symptoms of malarial fever.

Study Design

This is a Prospective study conducted in Institute of Pathology Rajeev Gandhi Government General Hospital Chennai.

Sample Size

The sample size (N) used in this study were 200 test sample from patient with clinically suspected malarial fever .

Out of which 60 samples were smear positive for malarial parasite and 140 samples were from smear negative for malarial parasite.

Ethical Approval

The ethical clearance for the study was obtained from the ethical committee before starting the study .

Patient consent was not required in this study since only random blood samples with clinically suspected for malaria was taken for study without compromising the quality check in collection, storage and interpretation.

Sampling Techniques

Sample Collection

2ml of EDTA sample was used for making a thick blood film and thin film to look for malarial parasite and identification of species and stage of parasite.

Test Sample

The Random test samples were obtained from 200 clinically suspected malaria fever patients from various inpatient wards and outpatient basis during the study period from 1st april 2018 to 1 st october 2019 (1.5 year)

Sample Processing

All sample collected were processed and maintained under aseptic condition and stored under correct temperature if not used immediately. Samples were examined on the same day at the earliest possible time and the reports were documented in the study..

Peripheral Thick Blood Film, Making And Staining Procedure:

1. A drop of whole blood was placed at the centre of a clean grease free glass slide.
2. Blood was spread uniformly to cover an area of 15×15mm.
3. The film allowed to air dry.
4. Once dried it is ready for staining.

Giemsa Stainig Method (USED FOR THICK SMEAR STAINING)[26]

1) A prepared thick blood film was covered with giemsa stain and allowed to act for 30 minute.
2) The stain was washed with tap water and it was allowed to air dry.

In our study we used Giemsa stain as per WHO guidelines

After staining is over look for malarial parasite density in oil immersion field in thick smear . look for parasites and found count them as per WHO protocol.However species identification is not possible in thick smear one has to rely on thin smear for the same.

Peripheral Thin Blood Film And Staining Procedure: (USED FOR THIN SMEAR STAINING)

1) A drop of whole blood was placed on the end of glass slide.
2) Thin smear is made with the help of spreader
3) The film was allowed to air dry by waving the slide back and front.
4) It was allowed to air dry and ready for staining.

Leishman Staining Method (In our study we used leishman stain for thin smear)

1) A prepared thin blood film was covered with leishman stain and allowed to act for 2 minutes.
2) The stain on the film was diluted with twice the volume of stain pH 6.8 buffered water for 8 minutes
3) The stain was washed with tap water and it was allowed to air dry.

Leishman stain was used in our study to look for malarial parasite species identification and their stages of development . In Thin smear apart from looking for malrial parasite, DC is also attempted to verify the Automated analyser generated reports and verified to prevent erranous reading by automated analyser there by maintaining Strict quality control while interprating its reports manually.

To say a peripheral smear is negative for malaria one has to examine atleast 100 fields properly. To avoid inter observer variation a second individual can confirm the same. Expert observer opinion is sort for in rare cases if needed. in this way false positive or false negative can be avoided.

Malaria Parasite Detection Method [27]

1) A drop of immersion oil was placed around the edge of the stained dried thick blood film.
2) The slide was then mounted unto the stage of microscope and first examined using 40x objectives. The objective was then changed to 100x.
3) The malaria parasites were examined and the approximate numbers of parasites were recorded.
4) In our study we used + signs to Grade the severity of parasitemia (Alternate method) by fitting in the Parasitic count as per WHO calculation.

   1+- mild parasitemia between 0-40 Parasites /micro litre

   2+ - mild parasitemia between 41-400 parasites /micro litre

   3+-Moderate parasitemia between 401 to 4000 parasites / microlitre

   4+ -Severe parasitemia more than 4000 parasites/ microliter

Most of the cases in our study were in moderate parasitemia and few cases were mild parasitemia. One case of very severe parasitemia was reported with smear positive for falciparum malaria

Apart from the above method WHO formulae for calculating parasitic index is applied in the study and the different haematological parameters with respect to RBC, WBC and PLATELETS were compared with degree of parasitemia and correlated.

Meanwhile the remaining received sample in EDTA tube is run in the SYSMAX 5 part Automated analyser SYSMAX XN1000 machine and also Sysmax 3 part analyser which used in our study.The Sample is feeded to the 5 Part analyser and with in few minutes all the CBC parameters along with histogram and scattergram is displayed in the output screen.

Though Scattergram findings are significant with respect to malaria, it was out of scope in the current study hence not documented instead wbc histogram obtained from the sample sample from 3 part analyser is studied for wbc flag instead of WBC scattergram abnormalities.

FIG 21. SYSMAX XN 1000 USED IN OUR STUDY

The CBC Parameters included RBC Parameters like RBC count, HGB, HCT , MCV, MCH , MCHC , RDW-SD, RDW-CV were recorded for all 200 samples.WBC parameters like WBC count, Differential count for neutrophils ,lymphocytes, monocytes, eosinophils, monocytes , basophils were recorded for all 200 samples.

Platelet parameters like platelet count, MPV, PDW,PCT, P LCR were recorded for all samples.N RBC percertage recorded in all 200samples.The 200 samples were examined by thick smear, thin smear by atleast two observer .Expert opinion is sort for in suspicious cases to avoid bias in the diagnosis.QBC is done in cases with low parasitic density as seen in thick smear.Based on the interpretation of blood smear by gold standard test (microscopy)

The 200 Samples were divided into two groups based on positivity of malarial parasite in blood smear. First group included 60 smear positive cases for malaria confirmed by gold standard microscopy and QBC cases. Second group included 140 smear negative cases for malaria confirmed by gold standard microscopy and QBC cases.

Tabulation were done as two groups. One is malaria group other is non malaria group. The different results for all 200 samples generated by the automated analyser were entered in the microsoft word excel sheet. Each parameter among the malaria and non malaria group is compared with standard

statistical tool and P value is derived and significance for each parameter is noted and compared .

Like wise among the smear positive 60 cases -it is further divided into 3 groups based on the severity of parasitemia as mild, moderate and severe and different blood parameters were again compared with in the malaria positive group using standard statistical tool and P value for each is derived and correlation between degree of parasitemia (mild, moderate, severe) with each hematological parameter is drawn.

Apart from hematogical parameters ,Histogram flags were studied in RBC WBC and PLATELET histogram.RBC histogram findings like shift to right, shift to left, double peak, broadening of the width of the RBC histogram which is non specific for malaria is noted and documented in all 200 samples.Like wise WBC histogram flags Like WL flag and flag in mixed population were noted and documented in all 200 samples.WL flag in WBC histogram which is highly suspicious for malarial parasite is noted and documented in all 200 samples.NRBC in all 200 samples is seen to avoid false positive peak before 60 FL.Platelet histogram flags are very common and non specific is noted in malaria positive cases also. Sensitivity, specificity, accuracy, PVV, NVV is noted for WBC histogram flags suspicious for malaria.

TRUE POSITIVE (TP)- Both smear positive for malarial parasite and positive in WBC histogram flags WL flag with or without flag in mixed region.

FALSE NEGATIVE (FN)- Smear positive for malarial parasite and negative inWBC histogram flags(abnormal peak WL FLAG)

TRUE POSITIVE & FALSE NEGATIVE

- Indicate people actually having the disease

TRUE NEGATIVE(TN) - Smear negative for malarial parasite and negative in WBC histogram flags(abnormal peak WL FLAG)

FALSE POSITIVE (FP) - Smear negative for malarial parasite and positive in WBC histogram flags (abnormal peak WL LAG) Accuracy of the test is also documented.

TRUE NEGATIVE(TN) AND FALSE POSITIVE (FP)

-Indicate actual people without disease

STATISTICAL PARAMETERS

1) Descriptive statistics of the quantitative parameters in the study were expressed in/as Mean±SD.
2) The difference in quantitative data between the two groups (Malaria and Non-malaria) were analysed using independent samples t test.
3) The difference in quantitative data between more than two groups (thrombocytopenia grade or platelet density) were analysed using One-way ANOVA, followed by Scheffe's post hoc test to derive the results of pairwise comparisons (given as foot notes below all the ANOVA tests)
4) Difference in distribution of frequency between the groups (Malaria/Non-malaria) were analysed using chi-square tests.
5) Wherever applicable, the difference in considered statistically significant at $P<0.05$ (significance is set at 5%).

OBSERVATION AND RESULTS

RESULT

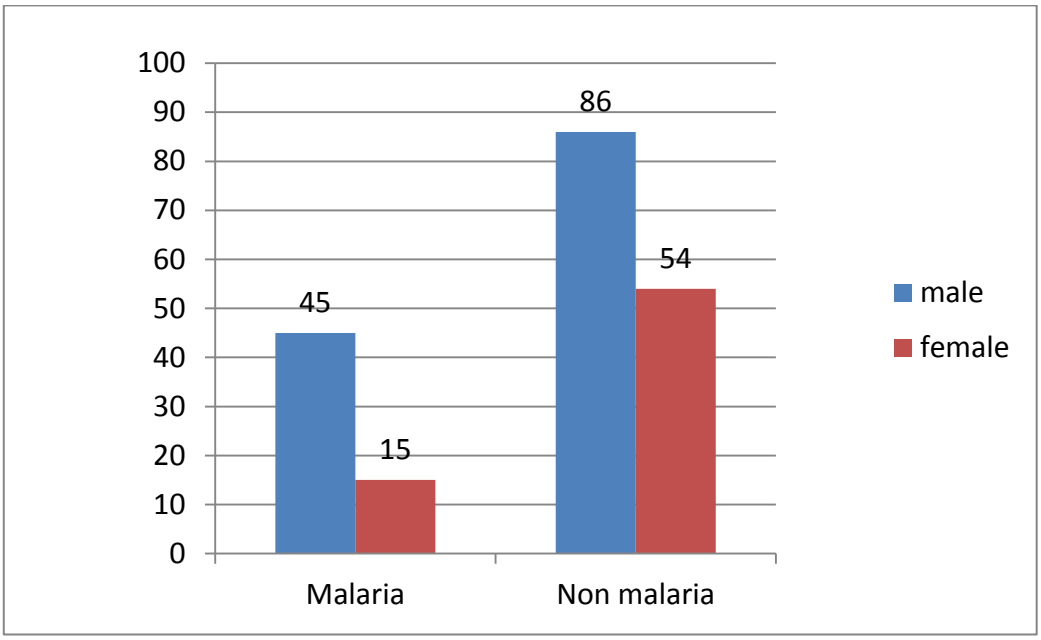
SEX WISE DISTRIBUTION

Among the 200 samples from patients from clinical symptoms of malaria fever131 were males 65.5% and 69 were females 34.5 %. Among the smear positive malaria confirmed cases of 60 samples . 45 were males (75%) and 15 (25%) were females.

TABLE 5:SEX WISE DISTRIBUTION OF MALARIA SUSPECT CASES

Group	Gender	Frequency	Percent
Malaria	Male	45	75.0
	Female	15	25.0
Non-malaria	Male	86	61.4
	Female	54	38.6

CHART 1 :SEX WISE DISTRIBUTION OF MALARIA SUSPECT CASES

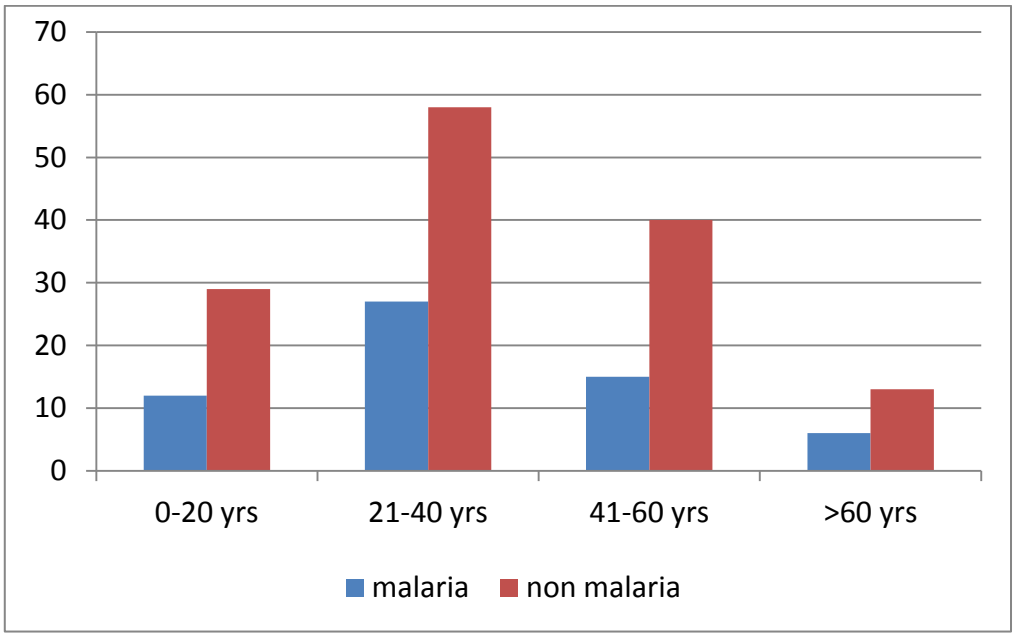


In our study male population more than female population.

TABLE .6 AGE WISE DISTRIBUTION OF CASES

	Malaria	Non Malaria
0-20 yrs	12	29
21-40 Yrs	27	58
41-60 Yrs	15	40
>60 Yrs	6	13

CHART.2 AGE WISE DISTRIBUTION OF CASES



Among the 200 malaria suspected samples majority fall under the category of 21-40 age group which accounts for 85 cases 42.5% followed by 41-60 age group constituting 45 cases 22.5%. Children and adolescents contribute 41 cases 20.5 % and elderly population constitute mere 9.5% in our study. Similar trend is followed in malaria positive cases also.

TABLE 7. STAGES OF PARASITE IN OUR STUDY

Malaria positive n=60	Trophozoite	Trophozoite +Ring Forms	Trophozoite +Schizont	All forms	Only Ring form
60	25	07	22	05	01(falciparum)

In the current study 59 cases reported for plasmodium vivax asexual stages of the hemoparasites.01 case was reported as plasmodium falciparum in which asexual form (Ring form) is noted. Gametocytes identified and is documented in one case reported as falciparum however gametes are not counted in calculating parasitic density.

Hematological parameters with respect to malarial parasite asexual stages in Erythrocytic Schizogony was not studied in the current study.But Changes in Hematological parameters with respect to parasitic density and severity were studied.

TABLE 8. SHOWING DEGREE OF PARASITEMIA IN THE SMEAR POSITIVE SAMPLE FOR MALARIA

TOTAL CASES	MILD	MODERATE	SEVERE	VERY SEVERE
60	4	48	7	1(FALCIPARUM)

The degree of parasitemia is documented as mild , moderate, severe based on parasitic density and its relation with various hematological parameters is compared .

RBC PARAMETERS IN MALARIA SUSPECT CASES

TABLE 9 . DESCRIPTIVE STATISTICS AND INDEPENDENT SAMPLES T TEST FOR COMPARING THE MEAN VALUES BETWEEN THE GROUPS (FOR RBC RELATED INDICES)

Variable	Group	N	Mean	SD	SEM	Mean difference	t	P value
RBC	Malaria	60	3.781	.885	.114	0.293	1.6	0.10
	Normal	140	4.074	1.25	.105			
HGB	Malaria	60	10.54	2.36	.304	0.95	2.2	0.025
	Normal	140	11.42	3.56	.300			
HCT	Malaria	60	31.99	6.88	.889	2.60	2.09	0.037
	Normal	140	34.60	10.62	.898			
MCV	Malaria	60	84.27	9.214	1.18	0.016	0.01	0.9
	Normal	140	84.85	15.10	1.27			
MCH	Malaria	60	28.36	2.972	.383	0.087	0.1	0.9
	Normal	140	28.48	5.916	.500			
MCHC	Malaria	60	32.87	1.585	.204	0.163	0.33	0.8
	Normal	140	33.27	5.329	.450			
RDW-SD	Malaria	60	48.64	7.177	.926	3.1	2.0	0.11
	Normal	140	45.50	14.49	1.22			
RDW CV	Malaria	60	15.64	2.164	.279	0.8	1.8	0.13
	Normal	140	14.84	3.829	.323			

Inference: The test shows that HGB and HCT alone varies significantly different among the two groups (lower for malaria positive). The other parameters were not significantly different.Anaemia was very common in the current study.

In Harool at al observed anemia in *falciparum* malaria to be 80% sensitive and 100% specific.Our study dealt mainly with vivax hence specificity cannot be as high as observed by Harool.[113]

TABLE 10. SEVERITY OF MALARIA IN MALARIA POSITIVE CASES

TOTAL CASES	Normal Male >13gm/dl Female >12gm/dl	Mild >10 gm <normal value for male and female	Moderate 8.5-10 gm/dl	Severe (8.5-6.5gm/dl)	Very Severe (<6.5gm/dl)
60	10	31	11	5	03

In our study malaria cases showed significant p value for Haemoglobin and Haematocrit between malaria positive and malaria negative cases.

TABLE 11: HAEMOGLOBIN VALUES BETWEEN MALARIA POSITIVE AND NON MALARIA CASES

	Haemoglobin		Chi-square	P value
	Anaemic	Non-anaemic		
Malaria	51	9	9.18	**0.002**
Non malaria cases	89	51		

Significant difference found: More patients with anaemia in malaria group.

TABLE12: RDW -CV VALUE BETWEEN MALARIA POSITIVE AND NON MALARIA CASES

	RDW-CV		Chi-square	P value
	Anisocytosis	Normal		
Malaria	37	23	7.4	**0.007**
Non malaria cases	57	83		

Significant difference found: More patients with Anisocytosis in malaria group. Hence anemia and anisocytosis is observed in malaria in the current study.

TABLE13: RBC SIZE VARIATION AND HISTOGRAM VARIATIONS AMONG MALARIA POSITIVE CASES

TOTAL CASES	**NORMOCYTIC RBC 80-96 FL**	**MICROCYTIC RBC <80 FL**	**MACROCYTIC RBC >80FL**	**DIMORPHIC RBC TWO POPULATION**
60	13RBC HISTOGRAM - NORMAL CURVE22%	**38**RBC HISTOGRAM SHIFT TO LEFT **63%**	7RBC HISTOGRAM SHIFT TO RIGHT 11.7%	2RBC HISTOGRAM WITH DOUBLE PEAK 3%

TABLE 14 .ONE-WAY ANOVA - COMPARISON OF RBC INDICES ACCORDING TO PARASITIC DENSITY

		Mean	SD	95% CI for Mean		F	P value
				Lower	Upper		
RBC	Mild	4.42	.55	3.72	5.11	2.385	0.06
	Moderate	3.79	.89	3.53	4.06		
	Severe	3.29	.92	2.43	4.15		
HGB	Mild	12.42	2.25	9.61	15.22	3.56	**0.035**
	Moderate	10.59	2.23	9.94	11.24		
	Severe	8.91	2.43	6.66	11.16		
HCT	Mild	36.60	6.07	29.05	44.14	3.68	**0.031**
	Moderate	32.04	6.72	30.09	34.00		
	Severe	26.24	7.25	19.53	32.95		
MCV	Mild	81.18	4.55	75.52	86.83	0.852	0.43
	Moderate	85.15	12.25	81.59	88.71		
	Severe	79.62	11.22	69.23	90.00		
MCH	Mild	28.00	2.18	25.28	30.71	0.16	0.85
	Moderate	28.13	3.80	27.02	29.23		
	Severe	27.28	3.58	23.96	30.60		
MCHC	Mild	33.64	1.11	32.25	35.02	5.28	**0.008**
	Moderate	32.83	1.73	32.33	33.34		
	Severe	35.04	1.79	33.37	36.70		
RDWSD	Mild	44.54	3.08	40.71	48.36	2.5	0.085
	Moderate	49.69	7.21	47.5973	51.78		
	Severe	44.57	7.30	37.8157	51.32		
RDWCV	Mild	14.54	1.12	13.1446	15.93	2.12	0.12
	Moderate	15.91	2.22	15.2718	16.56		
	Severe	14.48	1.73	12.8772	16.09		

Difference found between

In RBC : Mild vs severe (more in Mild)

IN HGB : Mild vs severe (more in Mild)

In HCT : Mild vs severe (more in Mild)

In MCHC : Moderate vs Severe group (More in Severe)

Over all Inference with the RBC parameters studied.

In our study with clinically suspected malaria samples showed significan p value of <0.001 for parameters like haemoglobin and haematocrit between malaria cases and non malaria cases which indicates anaemia.

Among the malaria positive cases (n=60) 31 had mild , 11 had moderate , 5 had severe and 3 had very severe anaemia which is expected in vivax positive malaria which is the majority population in our study.Among the severe anemia causes non of them required platelet transfusion indicating that bleeding manifestations and shock are not common in malaria unlike in dengue.

RDW-CV was significant between malaria and non malaria group p value of 0.007 . and 40 out of 50 smear positive cases showed high rdw cv indicating anisocytosis.

RBC indices like MCH MCHC MCV Showed no significant p value among clinically suspected malaria fever.

Out of 60 malaria positive cases 38 cases were microcytic RBC with shift to left , 7 cases showed macrocytic RBC. 2 Cases showed dimorphic features and 13 cases were normocytic rbc seen.

When the RBC parameters are compared with degree of parasitemia Hemoglobin , Hematocrit and MCHC showed significant p value with indicates there parameters reduced with severity of parasitemia which mean severe parasitemia cause severe anaemia due to increased parasitic load.

RBC count showed relatively reduced with **p value 0.09** when compared with degree of parasitemia.

RBC histogram showed shift to left(microcytic anemia) in in majority of cases 63.3%, shift to right in 11.6 % cases (macrocytic aneamia)and double peak (dimorphic)in 3.3% cases .21.6 % of cases showed normal rbc histogram curve..

WBC PARAMETERS IN MALARIA SUSPECT CASES

TABLE 15. DESCRIPTIVE STATISTICS AND INDEPENDENT SAMPLES T TEST FOR COMPARING THE MEAN VALUES BETWEEN THE GROUPS (FOR WBC RELATED INDICES)

Variable	Group	N	Mean	SD	SEM	Mean difference	t	P value
WBC	Malaria	60	4.87	1.89	.24	3.71	6.33	**<0.001**
	Normal	140	8.58	6.29	.53			
NEUT	Malaria	60	63.96	12.56	1.62	1.91	0.81	0.41
	Normal	140	62.05	19.96	1.68			
LYMPH	Malaria	60	22.25	9.79	1.26	1.76	0.97	0.32
	Normal	140	24.02	15.22	1.28			
MONO	Malaria	60	8.08	4.32	.558	0.45	0.57	0.56
	Normal	140	8.53	5.46	.462			
Eosino	Malaria	60	4.84	5.35	.690	2.58	3.14	**<0.001**
	Normal	140	2.26	3.62	.306			
BASO	Malaria	60	.173	.246	.031	0.14	1.8	0.068
	Normal	140	.320	.596	.050			

Inference: The test shows that WBC count and Eosinophil mean values varies significantly different between the two groups. (both values higher in Normal cases)

TABLE 16: COMPARISON BETWEEN MALARIA AND NON MALARIA GROUP FOR LEUCOPENIA

	WBC count		**Chi-square**	**P value**
	Leucopenia	Normal		
Malaria	27	33	9.9	**0.002**
Non malaria	32	108		

Significant difference found: More patients with Leukopenia in malaria group

Facer at al [115] Showed leukopenia in malaria cases . Our study shows the same finding.

TABLE 17. COMPARISON BETWEEN MALARIA AND NON MALARIA FOR MONOCYTOSIS

	Monocytes		**Chi-square**	**P value**
	Monocytosis	**Normal**		
Malaria	23	37	1.5	0.2
Non malaria	41	99		

No significance found between the two groups.

In a study by Halim at al [116] there was marked increase in monocyte count in individuals with malaria infection asmonoctes tend to become phagocytic in the presence of protozoa , bacteria or fungal infection. In our study population p value for monocytes were not significant .

TABLE 18.COMPARISON BETWEEN MALARIA AND NON MALARIA FOR EOSINOPHILIA

	Eosinophils		Chi-square	P value
	Eosinophilia	Normal		
Malaria	18	42	15.12	**<0.001**
Non malaria	12	128		

Significant difference found: More patients with Eosinophilia in malaria group

Pseudo eosinophilia is common in the study group with significant p value and is almost co relating with the degree of parasitemia.

- Sensitivity of pseudo eosinophilia (smear positive)in our study is 19/60 x 100 =31.66%.
- Specificity of pseudo eosinophilia (smear negative)in our study is 135/140x100=96.42%

Pseudoeosinophila which is common in malarial infection which is manifesed by spurious increase of eosinophil count. [117]

Table 19. COMPARISON BETWEEN MALARIA AND NON MALARIA FOR LYMPHOCYTOSIS

	Lymphocytes		Chi-square	P value
	Lymphopenia	**Normal**		
Malaria	34	26	5.137	**0.023**
Non malaria	55	85		

Significant difference found: More patients with Lymphopenia in malaria group.

TABLE 20.COMPARISON BETWEEN MALARIA AND NON MALARIA FOR NEUTROPENIA

	Neutrophils		Chi-square	P value
	Neutropenia	Normal		
Malaria	2	58	2.53	0.111
Non malaria	14	126		

No significant difference found between Neutropenia cases in malaria and non-malaria groups

TABLE 21.ONE-WAY ANOVA - COMPARISON OF WBC INDICES ACCORDING TO PARASITIC DENSITY

		Mean	SD	95% CI for Mean		F	P value
				Lower	Upper		
WBC	Mild	7.0660	2.09454	4.4653	9.6667	4.3	**0.018**
	Moderate	4.7548	1.74740	4.2474	5.2622		
	Severe	4.1857	1.99194	2.3435	6.0280		
NEUT	Mild	60.4000	7.39324	51.2201	69.5799	0.84	0.43
	Moderate	63.6083	13.22168	59.7692	67.4475		
	Severe	69.3000	10.58127	59.5140	79.0860		
LYMPH	Mild	20.7800	11.03889	7.0734	34.4866	0.68	0.509
	Moderate	22.8750	9.99663	19.9723	25.7777		
	Severe	18.4000	7.71924	11.2609	25.5391		
MONO	Mild	8.6600	5.80500	1.4521	15.8679	0.599	0.55
	Moderate	8.2667	4.31609	7.0134	9.5199		
	Severe	6.4143	3.50496	3.1727	9.6558		
Eosino	Mild	9.6200	14.67777	-8.6049	27.8449	2.41	0.09
	Moderate	4.2750	3.63994	3.2181	5.3319		
	Severe	5.2571	3.20565	2.2924	8.2219		
BASO	Mild	.1600	.05477	.0920	.2280	0.715	0.49
	Moderate	.2523	.25720	.1776	.3270		
	Severe	.1571	.20702	-.0343	.3486		

Significant difference in WBC count

Mild vs moderate (mild is higher)

Mild vs severe (mild is higher)

TABLE 22.NRBC IN MALARIA SUSPECT CASES

NRBC	Malaria	60	.119	.1507	.019	0.54	1.1	*0.45*
	Non malaria cases	140	.662	5.665	.478			

In the WBC histogram the dotted line before the lymphocytic peak included NRBC.It causes the Same WL flag like in malaria infected parasites.

In RBC histogram NRBC causes additional peak in rbc curve.[119]In our study NRBC was not significant to interfere with the results.

FLAGGING IN WBC HISTOGRAM (USING 3 PART ANALYSER)

In the current study 57 cases were positive for malaria in both smear and histogram and considered true positive. Histogram showed both abnormal WBC peak and Flags suspicious for malaria.WL flag before Lymphocyte population (LD) along with or without wbc histogram flag due to mixed population in WBC histogram in 3 part analyser is suspicious of malaria.All the 57 cases which are smear positive showed such peak with or without abnormal platelet histogram.NRBC was normal in all the 57 cases which causes false flag WL before lymphocytic population is ruled out.

In our study 3 cases were negative on histogram but found positive on peripheral smear examination may be due to machine error which is possible inspite of strict quality control in diagnosis they constitute false negative

cases.113 cases were negative in both peripheral smear and histogram hence considered true negative.

Shamsi TS et al[120]enumerates the causes of WL flag as the presence of nucleated red blood cells, giant platelet, small platelet clumps, moderate number of target cells or polychromatophilic RBCs and malarial parasites.Sun et al. MalarJ[121] showedinfected RBC' was used to analyse the diagnosis performance in patients with malaria, as well as routine parameters and suspect flagsmalaria infected RBC are osmotically resistant to lysis due to multiple proteins play a role in generating increased rigidity of *Plasmodium*infected erythrocytes, that would result in resistance to lysis [122][123]

Trophozoites and schizonts of *P. falciparum* are rarely seen in the peripheral blood of infected patients. The ring and the gametocyte stage are the most commonly seen in a peripheral blood smear. However, the ring and the early trophozoites of *Plasmodium* cannot be detectedby automated analysers, the main reason possibly being the small size and low nucleic acid content. While in vivax the rbc size increased as seen by increase in RDW -CV in the reported cases along with mature forms of erythrocytic schizogony hence WL peak in histogram is highly suspicious for malaria.

In our study it was samples were exclusively positive for vivax unlike one sample which was positive for falciparum.NRBC in our study was not significant which can show abnormal WBC flag.57 Among 60 cases of malaria positives showed Abnormal WL flag . Falciparum case didnot show the WL flag for the

same reason because on peripheral smear only ring forms were seen along with gametes

TABLE 23. Sensitivity and specificity of Determined threshold peak (>2.2mm)in WBC histogram WL FLAG in 3 part histogram .Result for Abnormal WBC peak in malaria (n=200)

FLAG	Malaria smear positive cases n=60 (with actual disease)	Fever control - smear negative for malaria n=140 (without actual disease)
Abnormal WBC FLAG in 3 part analyser	57 (TP)	27 (FP)
Absent Abnormal WBC FLAG in3 part analyser	03(FN)	113(TN)

ABNORMAL WBC FLAG INCLUDES WL FLAG WITH OR WITHOUT FLAG IN MIXED POPULATION OF WBC HISTOGRAM

NRBC is not significant in the current study to cause false positive WL flag before LD in wbc histogram

Sensitivity (people with actual disease) = TP/TN+FP = 57/57+3= 95%

Specificity (people without actual disease) = TN/TN+FP=80.71%

PLATELET PARAMETERS IN MALARIA SUSPECT SAMPLES

TABLE 24. Descriptive statistics and independent samples t test for comparing the mean values between the groups (for platelet related indices)

Variable	Group	N	Mean	SD	SEM	Mean difference	t	P value
PLT	Malaria	60	54.70	39.23	5.06	122.4	11.7	**<0.001**
	Normal	140	177.13	108.15	9.14			
RDW-SD	Malaria	60	48.64	7.177	.926	3.1	2.0	0.11
	Normal	140	45.50	14.49	1.22			
RDW CV	Malaria	60	15.64	2.164	.279	0.8	1.8	0.13
	Normal	140	14.84	3.829	.323			
PDW	Malaria	60	14.44	7.242	.934	1.71	1.6	0.1
	Normal	140	12.73	5.081	.431			
MPV	Malaria	60	10.42	4.872	.629	0.12	0.17	0.83
	Normal	140	10.30	3.374	.286			
P CLR	Malaria	60	36.96	18.83	2.43	5.1	1.9	0.06
	Normal	140	31.86	13.29	1.12			
PCT	Malaria	60	.049	.0473	.006	0.14	12.1	**<0.001**
	Normal	140	.190	.1175	.009			
NRBC	Malaria	60	.119	.1507	.019	0.54	1.1	0.45
	Normal	140	.662	5.665	.478			

Inference: The test shows that Platelet count and PCT mean values varies significantly different between the two groups. (Platelet and PCT values higher in Normal cases)

TABLE 25. COMPARISON BETWEEN MALARIA AND NON MALARIA FOR THROMBOCYTOPENIA

	Platelets		Chi-square	P value
	Thrombocytopenia	Normal		
Malaria	59	1	53.64	**<0.001**
Non malaria cases	60	80		

Significant difference found: More patients with Thrombocytopenia in malaria group.

TABLE 26. SEVERITY OF THROMBOCYTOPENIA AMONG SMEAR POSITIVE MALARIA CASES.

Total smear positive cases	Normal	Mild	Moderate	Severe <50000	Severe <20000
60	3	5	16	36	04

TABLE 27.One-way ANOVA - Comparison of platelet indices according to parasitic density

		Mean	SD	95% CI for Mean		F	P value
				Lower	Upper		
PLT	Mild	124.8000	49.17520	63.7409	185.8591	13.7	0.001
	Moderate	51.0417	32.53146	41.5955	60.4878		
	Severe	29.7143	17.37541	13.6447	45.7839		
RDWSD	Mild	44.5400	3.08107	40.7143	48.3657	2.5	0.085
	Moderate	49.6913	7.21123	47.5973	51.7852		
	Severe	44.5714	7.30473	37.8157	51.3272		
RDWCV	Mild	14.5400	1.12383	13.1446	15.9354	2.12	0.12
	Moderate	15.9167	2.22073	15.2718	16.5615		
	Severe	14.4857	1.73918	12.8772	16.0942		
PDW	Mild	14.8600	3.47174	10.5493	19.1707	7.2	0.002
	Moderate	17.3708	4.04143	16.1973	18.5443		
	Severe	10.6714	7.15861	4.0508	17.2920		
MPV	Mild	11.1800	1.91755	8.7990	13.5610	6.1	0.004
	Moderate	12.7563	1.16105	12.4191	13.0934		
	Severe	9.4429	6.58834	3.3497	15.5361		
PCLR	Mild	35.6600	12.19397	20.5192	50.8008	4.5	0.015
	Moderate	45.5583	8.04693	43.2217	47.8949		
	Severe	34.1857	23.93425	12.0502	56.3212		
PCT	Mild	.1060	.06387	.0267	.1853	7.8	0.001
	Moderate	.0544	.04151	.0423	.0664		
	Severe	.0100	.01291	-.0019	.0219		
MRBC	Mild	.2040	.34355	-.2226	.6306	1.1	0.32
	Moderate	.1169	.12753	.0798	.1539		
	Severe	.0729	.09394	-.0140	.1597		

Significant difference is found in

In Platelet count

Mild vs moderate: (Mild is higher)

Mild vs severe: (Mild is higher)

In PDW

Moderate vs severe (Moderate is higher)

In MPV

Moderate vs severe (Moderate is higher)

In PCLR

Moderate vs severe (Severe is higher)

In PCT

Mild vs moderate ((Mild is higher))

Moderate bs severe (Moderate is higher).

Among the malaria suspect cases platelet parameters like platelet count and plateletcrit showed significant p value of <0.001.

The same platelet count and plateletcrit value showed a significant p value of <0.001 when compared with degree of parasitemia which means thrombocytopenia is very severe in severe parasitic density in malaria.Apart from platelet count, MPV p value -0.004 , PCLR p value 0.015 and PDW p value 0.002 showed significant p value when compared with degree of parasitemia among the malaria positive cases .

Out of 60 malaria positivecases 5 samples showed mild thrombocytopenia, 16 cases showed moderate thrombocytopenia while 36 cases should severe

thrombocytopenia.3 cases near normal platelet count.Among the 36 cases of severe thrombocytopenia 5 cases showed platelet count which included one case of falciparum malaria reported in our study.

Hence it is concluded from the platelet parameters that Thombocytopenia being the strong predictor of malaria.Aarti et al., encountered similar findings of reduction in platelet count with resultant thrombocytopenia in 24 of 27 cases infected with P. Vivax infection in India [124] [125]

TABLE 28. PLATELET HISTOGRAM IN MALARIA SUSPECT CASES

parameters	Plasmodium vivax N=60 Smear positive	Febrile control N=140 Smear negative	Remarks
Flag related to platelet(Abnormal platelet distribution)	All 59cases(TP) Absent in one case(FN)	Present in 33 cases(FP) and absent in 107 cases	N rbc not significant in our study

A sensitivity of 98.33 % and specificity of 76.42 % is obtained in our study. The cause for low specificity may be due to most of the samples collected in the monsoon season when dengue is endemic to the zone of study like malarial infection.

DISCUSSION

DISCUSSION

In the present study we observed thrombocytopenia with anemia and leucopenia along with pseudo eosinophilia was very common among the smear positive cases of malaria from among the over all sample of suspected malarial fever.Anemia was very common in our study.Supported by other studies.[114]

In our study with clinically suspected malaria samples showed significant p value of <0.001 for parameters like haemoglobin and haematocrit between malaria cases and non malaria caseswhich indicates anaemia.

Among the malaria positive cases (n=60) 31 had mild , 11 had moderate , 5 had severe and 3 had very severe anaemia which is expected in vivax positive malaria which is the majority population in our study.

Among the severe anemia cases none of them required platelet transfusion indicating that bleeding manifestations and shock are not common in malaria unlike in dengue.

RBC indices like MCH MCHC MCV Showed no significant p value among clinically suspected malaria fever.When the RBC parameters are compared with degree of parasitemia.Hemoglobin , Hematocrit and MCHC showed significant p value with indicates there parameters reduced with severity of parasitemia which mean severe parasitemia cause severe anaemia due to increased parasitic load.

RBC count showed relatively reduced with pvalue 0.09 when compared with degree of parasitemia.Microcytic hypochromic anemia was common in our study.RBC histogram showed shift to left(microcytic anemia) in in majority of cases 63.3%, shift to right in 11.6 % cases (macrocytic aneamia)and double peak (dimorphic)in 3.3% cases.21.6 % of cases showed normal rbc histogram curve.

Hence it has been concluded that Anemia is strong indicator for malarial infection in the current study.Maina *et al*. have described that these parameters have sensitivity and specificity of 80% and 84%, respectively [128].

In Harool at al[113]observed anemia in *falciparum* malaria to be 80% sensitive and 100% specific. Our study dealt mainly with vivax hence specificity cannot be as high as observed by Harool.

Among the malaria suspect blood samples ,WBC parameters like wbc count and eosinophil count showed significant p value of <0.001 between malaria positive and non malarial fever group.which means leucopenia and eosinophilia was significant in the study group of smear positive cases when compared with smear negative cases.

Majority of the studies shows leucopenia like our study which should significant p value for malaria cases and was degree of leucopenia was propotional to the degree of parasitemia.[115]

When among the clinically suspected malarial fever samples lymphopenia was significant with the p value of 0.023 between malaria positive and non malaria cases which means lymphopenia significant among malaria positive cases. However lymphopenia was not corelating with severity of parasitemia.Pseudoeosinophila which is common in malarial infection which is manifesed by spurious increase of eosino phil count. [126]

The FLAG in mixed population of F2 region in WBC histogram in 3 part analyser one should suspect pseudo eosinophilia or monocytosis. These pseudo Eosinophils are none other than the hemozoin ingested neutrophils. Out of 60 smear positive cases 20 had elevated count above cut off in eosinophil count. On manual examination 19 of them showed normal eosinophil count.

One sample had eosinophil percentage of 35 % confirmed by microscopy. The results comparing severity of parasitemia shows severe parasitemia has more eosinophil count than moderate level. Due to one case among 5 cases of mild parasitemia showing true eosinophilia in excess of 35% because of which p value for pseudoeosinophilia was not highly significant among the malaria positive samples based on severity of eosinophilia.

Excluding that sample might have given the p value in this case which is in accordance with literature review. Hence eosinophilia in malaria suspected cases should be reviewed for pseudoeosinophilia by manual microscopic count if possible. True eosinophilia has to be ruled out because in any parasitic infection true eosinophil count too can be elevated just as we saw one case with true

eosinophilia in our study.Sensitivity of pseudo eosinophilia (smear positive)in our study is 19/60 x 100 =31.66%

Specificity of pseudo eosinophilia (smear negative)in our study is 135/140x100=96.42%. Several authors have described pseudoeosinophilia or abnormal WBC scattergrams as a result of hemozoin-containing neutrophils using the Sysmex XE-2100 analyzer**.** [86-88]

Hemozoin in WBC seems to be a sensitive indicator of prognosis, an automated hematology analyzer may offer a new way to assess disease severity[89]In this study, the overall sensitivity (46.2%) and specificity (99.7%) of the Sysmex XE-2100 analyzer for the detection of malaria were lower than in the previous study of South Korea (69.4% and 100%, respectively). [87]

In several studies, the percentage of hemozoin-containing WBCs varied according to populations, age, and disease activity. 13–15 A remarkable reduction in sensitivity in this study was probably caused by the presence of younger and fewer circulating parasites, severity of infection, and host immunity factors, 16–18 which produced less hemozoin and were below the analyzer detection limits.

Our study showed sensitivity of 31.66 % and high specificity of 96.42% for psedoeosinophilia.The low sensitivity can be explained by the study presence of younger and fewer circulating parasites and less severe infection in the current study involving only eight cases out of 60 smear positive cases causing severe parasitemia which accounts for just 13.3 % of total malaria positive cases.

Most of the cases in the current study are moderate parasitemia (78.3%) cases.only 6.6 % cases had mild parasitemia.Hence moderate and severe parasitemia is associated with pseudoeosinophilia. A more percentage of cases in severe parasitemia Which could have generated more hemozoin could have given a better sensitivity in the current study.

Sensitivity of pseudo eosinophilia (smear positive)in our study is 19/60 x 100 =31.66%. Specificity of pseudo eosinophilia (smear negative)in our study is 135/140x100=96.42%. Lymphopenia was common in our study.

Berens-Riha N , Kroidl I, Schunk M, Alberer M, beissener M, pritsch M, et al.showed malaria causes lymphocytopenia and increase in neutrophil count.(Neutrophila)[118]No significant p value obtained for monocytes , neutrophils,basophils in the current study which involved majority of plasmodium vivax only.

NRBC was not significant in the study which some times falsely elevates the WBC count spuriously.nRBC can cause abnormal WL flag in wbc histogram.Since n rbc was not significant in the study false positive reading were reduced to minimum.

Histogram showing WL Flag with our without Abnormal flag in Mixed cell population zone in wbc histogram is highly suspicious for malarial infection. The mixed population contains hemozoin ingested monocytes , true eosinophils along with neutrophils which ingested hemozoin pigments which falls in

eosinophil region there by falsely increasing the count of eosinophil in the WBC counter.Finally among WBC parameters Leucopenia and pseudoeosinophilia is strong predictor of malaria in our study.Comparison with other studies WBC flags in histogram for malaria.

TABLE 29. COMPARISON OF OUR RESULTS WITH OTHER STUDIES.

Studies	Sensitivity	Specificity
Present study	**95%**	**80.17%**
Thomas (Portugal) et al study [127]	72%	98%
Aminder singh et al Study[128]	98%	94%
Sysmex 2100 et al Study[129]	46.2%	99.7%
Briggs et al study [130]	98%	94%
Mandelow et al study [131]	72%	98%

Platelet abnormalities in malaria are both qualitative and quantitative. Low platelet count emerged as the strongest predictor of malaria, a previous observation which we confirmed [132]Findings of thrombocytopenia with anemia is an important clue to the diagnosis of malaria in patients of acute febrile illness and this is in agreement with the findings of studies from Africa[132-133]. In the current study, 87% of the patients suffering from malaria showed some degree of thrombocytopenia. These figures are comparable to the observations of previous studies, in which the authors found thrombocytopenia in 59% to 71% of the patients [134].

Thrombocytopenia has been postulated to occur secondary to peripheral destruction, splenic sequestration, and platelet consumption secondary to DIC, but none of our patients showed any bleeding tendencies despite very low platelet counts. The presence of cellular bone marrow in some cases of malaria suggest that thrombocytopenia is not likely to be secondary to marrow failure; rather, the immunemedicated destruction of platelets has been postulated as the cause of thrombocytopenia [135]

Qualitative abnormalities of platelets are also found in malariainfected patients. The most commonly observed abnormality is the formation of giant platelets which helps in releasing premature megakaryocytes secondary to thrombocytopenia [136].

Among the malaria suspect cases platelet parameters like platelet count and plateletcrit showed significant p value of <0.001.The same platelet count and plateletcrit value showed a significant p value of <0.001 when compared with degree of parasitemia which means thrombocytopenia is very severe in severe parasitic density in malaria.Apart from platelet count , mpv p value -0.004 , PCLR p value 0.015 and PDW p value 0.002 showed significant p value when compared with degree of parasitemia among the malaria positive cases .

Out of 60 malaria positive cases 5 samples showed mild thrombocytopenia, 16 cases showed moderate thrombocytopenia while 36 cases should severe thrombocytopenia.3 cases near normal platelet count.

Among the 36 cases of severe thrombocytopenia 5 cases showed platelet count which included one case of falciparum malaria reported in our study.Hence it is concluded from the platelet parameters that Thombocytopenia being the strong predictor of malaria. Aarti et al., encountered similar findings of reduction in platelet count with resultant thrombocytopenia in 24 of 27 cases infected with P. Vivax infection in India [124-125]

A retrospective study by Chandra et al., in 334 cases of acute malaria in Uttarkhand state caused by P. vivax, P. falciparum and dual infection had thrombocytopenia with higher Mean Platelet Volume (MPV). The authors concluded that rise in MPV as more sensitive marker of P. vivax infection while Platelet Distribution Width (PDW) was found to be sensitive for P. falciparum infection. [137]

We observed similar Significant P value for MPV as well as PDW in the study population of smear positive cases of malaria involving majority of vivex cases (98%).Only one case of falciparum was reported in the current study hence no conclusion can be drawn regarding platelet indices.

In an another study by Chandra et al., an increased MPV was observed in malaria patients in which the pathogenesis of thrombocytopenia was due to increased peripheral destruction (hyperdestuctive) while decreased MPV was attributed to bone marrow disease as a result of hypoproductive state [138]An increase in MPV was higher in vivax infected 19 cases (28.78%) in the present series.

In our study 44 cases 74.57% of malaria positive cases showed increased MPV.All these data show most of the cases with thrombocytopenia in our study showed hyderdestructive pattern though hypo productive state cannot be ruled out.Panasiuk et al., and Conte et al., found that Platelet-Associated IgG (PAIgG) is increased in malaria and is associated with thrombocytopenia [139-140].

Platelet Histogram showed

A sensitivity of 98.33 % and specificity of 76.42 % is obtained in our study. The cause for low specificity may be due to most of the samples collected in the monsoon season when dengue is endemic showing similar platelet histogram abnormality like abnormal distribution of platelets.

SUMMARY

SUMMARY

1. Microscopy is the gold standard for diagnosing malaria.
2. We have indeed made an attempt to diagnose malarial infection using changes in the hematological parameters following the infection that is automation based malaria detection.
3. With the results obtained from the various parameters using suitable statistical tool with significant p value its observed thatAnemia, leucopenia, thrombocytopenia, along with lymphopenia and pseudoeosinophiliaare positive predictor of malarial infection in Complete blood count which gives diagnostic clue to the clinician who suspect the fever to be of malarial origin with its classical presentation of chills and rigors.

Histogram Flags generated by Automated analyers come handy in diagnosing malaria.The WBC histogram and its flags specific for malaria include WL flag before Lower discriminator in the histogram along with or without flag in mixed population of wbc histogram which represents monocytes, eosinophils as well as pseudoeosinophils which are actually neutrophils which after ingesting hemozoin pigment falls in the region of mixed population region of histogram generating FLAG specific for malaria.

Platelet Histogram is also noteworthy to mention since thrombocytopenia emerged as strong predictor of malaria in the current study as well as in many previous studies.Platelet histogram showed abnormal platelet distribution in the histogram graph with sensitivity of 98.33% and specificity of 76.42%.

CONCLUSION

CONCLUSION

Any case with anaemia , leucopenia ,pseudoeosinophilia with thrombocytopenia along with abormal flag in wbc histogram specific of malarial infection and abnormal platelet histogram is highly suspicious for malaria infection especially in the endemic zone and remote rural areas where microscopy diagnosis, QBC and other invasive tests are not feasible to diagnose malaria to initiate emperical antimalarial treatment for the population under risk.

All these tests though not officially approved to diagnose malaria by WHO can be used to catch malaria in early stage in asymptomatic individuals also with the knowledge of these graphs & patterns paramedical staff, resident doctors, senior pathologists & even treating physicians might not miss malarial parasites even in the absence of a clinical request.

More slides will be reviewed to ensure no case is missed and delayed from initiating antimalarial treatment at the earliest at the primary level. With consistantly good sensitivity and specificity as shown by various previous studies. Its time to look into the practical implementation of such observation and get the diagnostic clue to diagnose malaria even if chills and rigor pattern of disease is not manifested.

STUDY LIMITATIONS

Study is confined only to the malaria suspect cases , normal control cases were not compared in the present study. The study involved plasmodium vivax(N=59) 98.33 % and the rest Plasmodium falciparum .(N=1)

The mixed infections and infection with ovale and malariae and its flags in histogram and blood parameters were not studied due to rarity of these cases in the area of study which is located in Chennai , Tamilnadu.The study showed more of mild to moderate parasitic load . Severe load is seen only in 8 out of 60 cases. which included one case of falciparum anaemia with very severe uncomplicated malaria with parasitic density of 42000/micro litre of blood.

Reticulocyte count was not studied in the present study which is usually low in malarial infections especially in vivax which infects new young RBC.We didnot consider the immune status of the individual either primary or acquired which might have interfered with the study results.Like wise Haemoglobinopathis like sickle cell anaemia and thalasemmia was not ruled out in the study population which might have interfered with our results.

Study is conducted in endemic zone for vivax in Chennai.It will be helpful to conduct such study else were in indian belt endemic for malaria were falciparum is prevalent which is the major cause of mortality and morbidity associated with malaria due to its very high parasitemia and acute hemolytic episode.5 part analyser used in this doesnot generate wbc histogram which is one of the pitfall of 5 part analyser instead they generate scattergarms which is not the part of our study which could have given even better sensitivity and specificity in the current study. 5 part analyser are very expensive and maintainance when compared to 3 part analysers can come handy in such situations which is inexpensive affordable, easy to handle .

FUTURE DIRECTIONS

In the current study only various hematological parameters and their histograms were studied in the study population of malaria suspect cases.Since Scattergram report is also generated in 5 part analyser using optical scatter principle it paves the way to incoporate even these scatter plots in different channels to diagnose malaria using scatter plot which co relates with abnormal wbc histogram with flags .

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IMAGES

THIN SMEAR

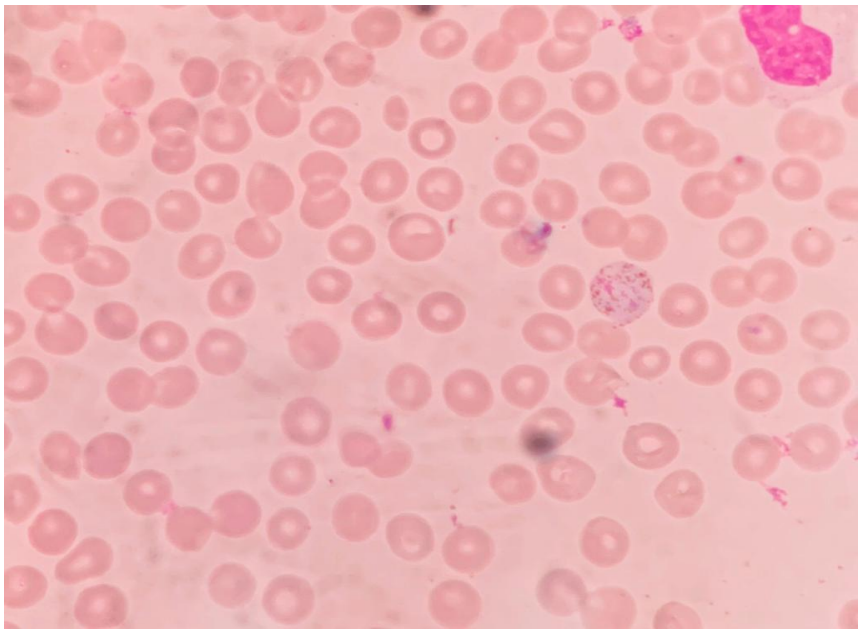

FIGURE 1. PS 15217/19 Thin Smear Plasmodium Vivax- Trophozoite

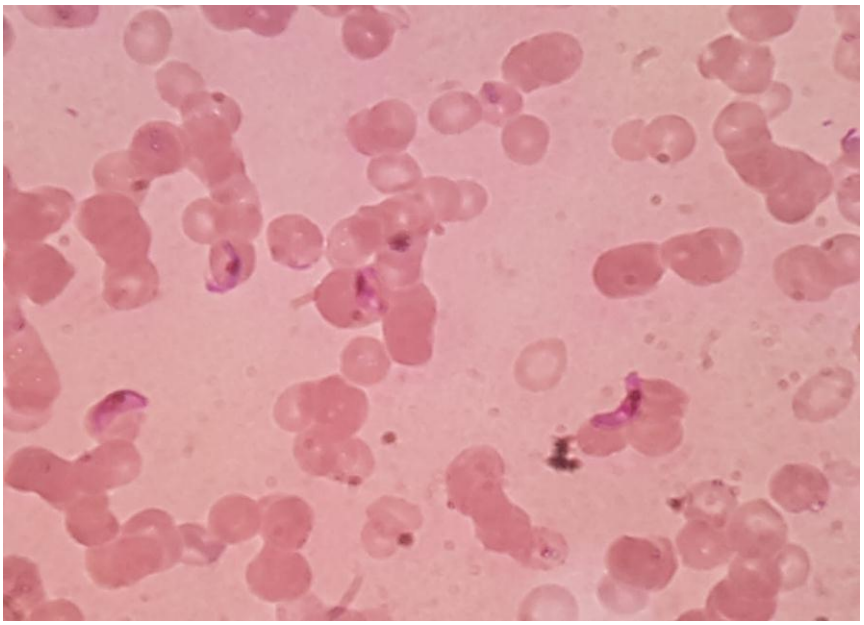

FIGURE 2. PS 5427/19 Thin Smear -Plasmodium Falciparum - Severe Parasitemia -Gametocytes noted.

THICK SMEAR

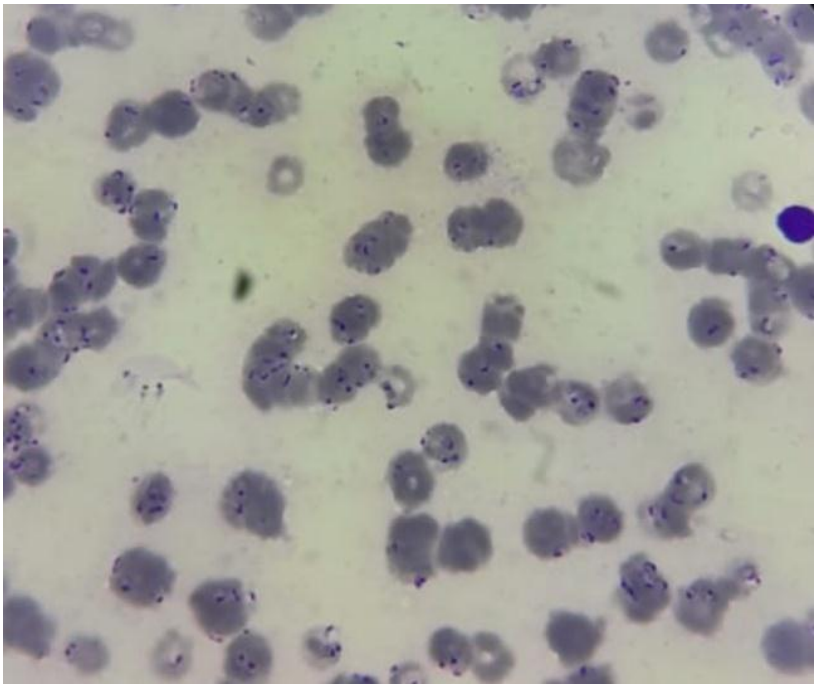

FIGURE 3. PS5427/19- Thick Smear- Ring forms noted in Plasmodium falciparum- Severe Parasitic Density.

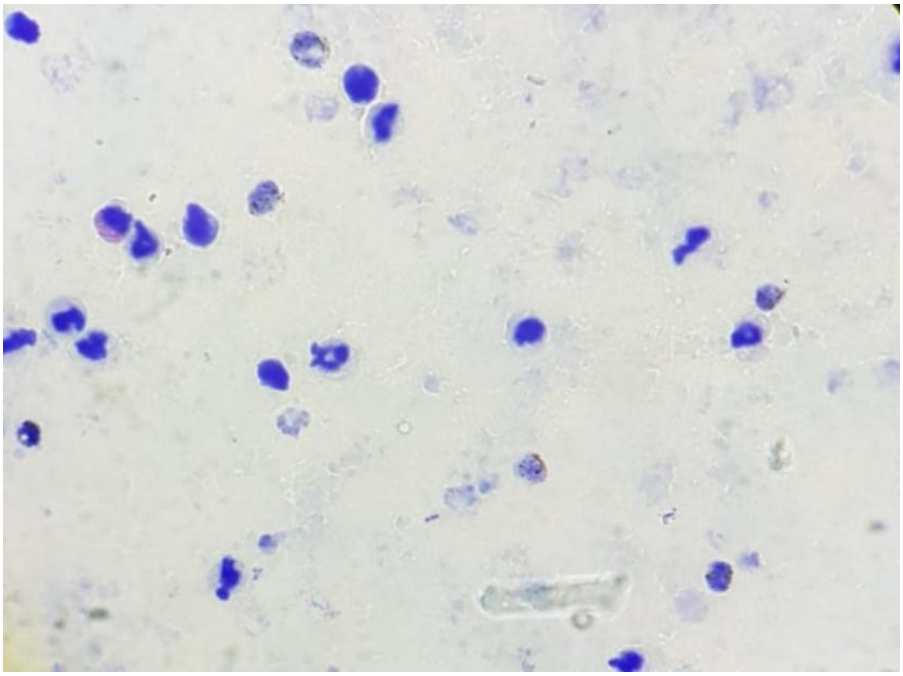

FIGURE 4 PS 14217/19. Thick Smear Plasmodium vivax – trophozite form -moderate parasitemia.

CBC REPORT

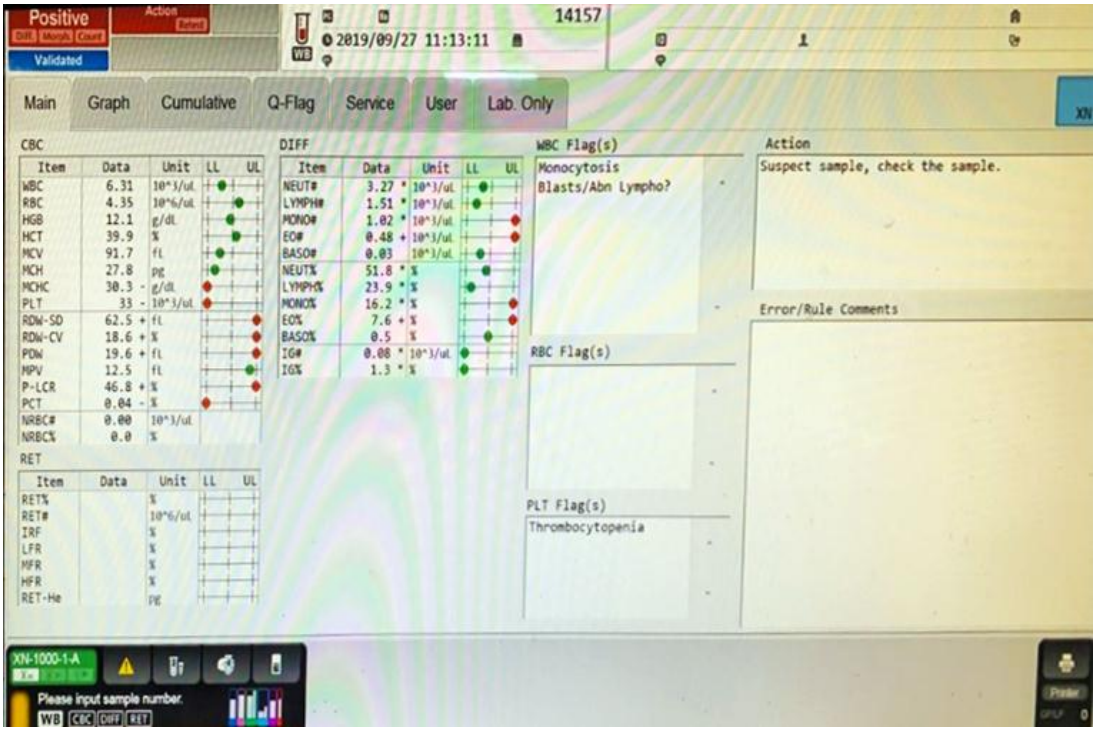

FIGURE 5 : Sample Report : CBC 14157/19-

Hematological Parameters in 5 Part Analyser Report in Vivax Infection

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ANNEXURE

MALARIA MICROSCOPY
STANDARD OPERATING PROCEDURE – MM-SOP-07A

1. PURPOSE AND SCOPE

To describe the procedure for properly staining malaria blood films with Giemsa stain. This procedure is to be modified only with the approval of the national coordinator for quality assurance of malaria microscopy. All procedures specified herein are mandatory for all malaria microscopists working in national reference laboratories, in hospital laboratories or in basic health laboratories in health facilities performing malaria microscopy.

2. BACKGROUND

A properly stained blood film is critical for malaria diagnosis, especially for precise identification of malaria species. Use of Giemsa stain is the recommended and most reliable procedure for staining thick and thin blood films. Giemsa solution is composed of eosin and methylene blue (azure). The eosin component stains the parasite nucleus red, while the methylene blue component stains the cytoplasm blue. The thin film is fixed with methanol. De-haemoglobinization of the thick film and staining take place at the same time. The ideal pH for demonstrating stippling of the parasites to allow proper species identification is 7.2.

Methods of staining

The two methods for staining with Giemsa stain are the rapid (10% stain working solution) and the slow (3% stain working solution) methods.

The rapid (10% stain working solution) method

This is the commonest method for staining 1–15 slides at a time. It is used in outpatient clinics and busy laboratories where a quick diagnosis is essential for patient care. The method is efficient but requires more stain. The need for speed justifies the additional cost.

The slow (3% stain working solution) method

The slow method is used for staining larger numbers of slides (≥ 20). It is ideal for staining blood films collected during cross-sectional or epidemiological surveys and field research and for preparing batches of slides for teaching. It is less appropriate when a quick result is needed. The slow method is less expensive than the rapid method because it requires much less stain (3% rather than 10% stain solution).

3. SUPPLIES AND MATERIALS

For the rapid (10% stain working solution) method

- Giemsa stain (10% solution) (See MM-SOP-04 for method of preparation);

- a small container or beaker for Giemsa working stain;
- absolute methanol, acetone-free;
- a Pasteur pipette with a rubber teat;
- a small container or beaker for methanol;
- a curved plastic staining tray, plate or staining rack;
- a timer;
- a slide-drying rack;
- a small electric hair-dryer;
- protective latex gloves, powder-free, disposable and
- Distilled or deionized water buffered to pH 7.2.

For the slow (3% stain working solution) method

- Giemsa stain (3% solution) (See MM-SOP-04 for the method of preparation);
- a small container for Giemsa working stain;
- absolute methanol, acetone-free;
- a Pasteur pipette with a rubber teat;
- a small container or beaker for methanol;
- staining troughs that can hold 20 slides placed back to back;
- a timer;
- a slide-drying rack;
- protective latex gloves, powder-free, disposable, and
- distilled or deionized water buffered to pH 7.2.

4. SAFETY PRECAUTIONS

1. Methanol (methyl alcohol) is inflammable and highly toxic if inhaled or swallowed; it can cause blindness and even death if swallowed in any quantity. Avoid contact and inhalation. When it is not in use, it should be stored in a locked cupboard.
2. Universal precautions – including use of relevant personal protective equipment such as gloves, safety glasses and a laboratory coat or gown – must be practised. See MM-SOP 11: General safety procedures in the malaria microscopy laboratory.

Quantitative Buffy Coat (QBC) PROCEDURE

Two methods, thick and thin blood smear microscopy are regarded as the "gold standard" for the diagnosis of malaria. Quantitative Buffy Coat is another direct and rapid test for diagnosis of malaria. It is based on acridine orange staining of centrifuged peripheral blood samples in a microhematocrit tube (QBC) and examination under UV light source (fluorescence microscopy).

The acridine orange stains all nucleic acid containing cells and the associated fluorescence is observable under blue-violet light through a microscope.

QBC Malaria Test is 5.5 to 7% more sensitive than Giemsa thick films. It can detect as little as 1 parasite per μL of blood and establish diagnosis earlier than thick film in 47% of low parasitemia (<10 parasites per μL) cases.

QBC is established as an effective tool for diagnosing blood parasites that cause malaria, filariasis and visceral leishmaniasis.

Principle:

Acridine orange binds deoxyribonucleic acids and ribonucleic acids. The malaria parasite binds acridine orange in the nucleus and the cytoplasm and emits green and red fluorescence when excited by blue light (at 460 nm) allowing the detection and examination of parasite morphology by fluorescent microscopy. The nuclei of the parasites emit yellowish green fluorescence whereas the cytoplasm exhibits bright red fluorescence. RBCs are not stained by the dye, hence remain inconspicuous under fluorescent light (dark background) while the brightly fluorescent parasites are easily seen. The outlines of stained parasites are well preserved and the general morphology is similar to that in specimens stained by the Giemsa stain.

Sample collection:

Blood sample can be collected in either capillary finger-prick or phlebotomy in a ethylenediamine tetraacetate (EDTA) containing vials.

About the QBC tube:

The QBC glass capillary tube (Becton Dickinson) is 75 mm in length and 1.677 mm in diameter. The tubes are internally coated with EDTA and heparin at the fill end and with acridine orange stain and potassium oxalate at the other end.

Procedure:

Draw samples of blood (55 μl) in to the QBC tube by capillary action.
Rotate the tubes for 10 seconds to dissolve the contained residues in the blood.
Insert a close fitting cylindrical insert or plastic float { having a specific gravity (1.055) i.e midway between that of plasma (1.028) and red blood cells (1.090)} inside a acridine orange-coated capillary tube.
Centrifuge the tubes for 5 minutes.
After gentrification blood components and malaria parasites separate based on density, and concentrate in distinct layers.

Note: The float by virtue of its density settles on top of the centrifuged packed red cells. It occupies 90% cross-sectional area of the tube which aids in the expansion of the centrifugally seperated cell layers. It is surrounded by three discernible and now measurable layers of the buffy coat.

Insert the centrifuged QBC Malaria test into the Paraviewer. Position the tube so the closure end extends over the depressed area of the holder.

The area surrounding the float just beneath the buffy coat was examined under oil immersion. Individual cells within this layer were easily seen by microscopy; the malaria parasites staining green (DNA) and orange (RNA) under blue-violet light.

The entire circumference of the tube was examined systematically while moving away from the buffy coat through the erythrocyte layer.

Each tube was examined until parasites were detected or for a maximum of 5 minutes.

If a sample contains P. falciparum malaria parasites:
Crescent shaped gametocytes (1) will appear near the interface of the lymphocyte/monocyte and platelet layers.

A small number of schizonts and mature trophozoites may appear in the granulocyte layer. Ring-shaped immature trophozoites will appear throughout the red blood cell layer, with a concentration near the interface with the granulocyte layer.
Other parasites species, including P. vivax, will also concentrate during centrifugation, but exhibit different characteristics.

MASTER CHART - MALARIA NEGATIVE SAMPLES

S. NO	ITEM NO.	Age/ Sex	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RDW-SD	RDW CV	PDW	MPV	P CLR	PCT	MRBC	NEUT	LYMPH	MONO	Eosino	BASO	WL FLAG WBC+/- MIXED FLAG	PLATELET FLAG	QBC
1	8879	20/M	6.26	3.35	8.70	26.00	77.60	26.00	33.50	16.00	44.10	15.90	18.80	12.00	46.30	0.02	0.20	82.00	11.80	4.20	0.00	0.20	PRESENT	PRESENT	NEGATIVE
2	15473	62/F	9.48	4.78	12.60	36.90	77.20	26.40	24.10	217.00	36.01	13.10	15.20	11.60	34.90	0.25	0.00	57.20	35.80	5.00	1.30	0.20	ABSENT	ABSENT	NEGATIVE
3	15474	56/M	7.58	4.84	14.01	41.40	85.50	29.01	34.01	358.00	40.90	13.20	9.60	9.30	18.10	0.33	0.00	49.30	37.90	9.10	2.20	0.30	ABSENT	ABSENT	NEGATIVE
4	15475	60/F	8.38	4.36	11.20	35.70	81.90	25.70	31.40	270.00	49.01	16.80	10.07	9.90	23.60	0.27	0.10	63.90	20.60	10.50	4.40	0.20	ABSENT	ABSENT	NEGATIVE
5	15493	40/F	41.48	0.46	2.60	7.60	165.20	56.50	34.20	118.00	38.00	14.40	14.20	11.50	34.60	0.14	67.00	59.90	24.20	4.80	0.10	0.50	ABSENT	ABSENT	NEGATIVE
6	15494	16/M	7.21	4.76	12.80	37.20	78.20	26.90	34.40	37.00	36.50	12.80	9.20	9.60	21.60	0.04	0.00	46.90	42.30	8.50	0.70	0.60	ABSENT	ABSENT	NEGATIVE
7	15759	55/M	18.90	3.23	9.20	27.70	85.80	28.50	33.20	109.00	53.00	17.20	22.06	13.80	55.00	0.15	0.50	89.40	6.60	1.80	1.40	0.10	ABSENT	PRESENT	NEGATIVE
8	15760	55/M	4.93	7.80	24.02	70.30	90.10	31.00	34.40	54.00	41.10	14.40	11.80	10.20	25.40	0.05	0.20	54.90	32.90	4.90	5.90	0.60	ABSENT	ABSENT	NEGATIVE
9	15763	58/M	13.24	4.27	12.30	35.30	82.70	28.80	34.80	228.00	44.50	15.30	17.40	12.60	34.90	0.29	0.00	84.20	5.80	8.80	0.00	0.20	ABSENT	ABSENT	NEGATIVE
10	15765	70/M	13.15	3.51	9.40	27.60	78.60	26.80	34.10	81.00	39.20	13.60	16.10	11.80	34.60	0.10	0.10	83.80	6.50	8.40	0.30	0.30	ABSENT	ABSENT	NEGATIVE
11	15771	F6	5.17	4.57	12.40	37.30	81.60	27.10	33.20	31.00	39.30	13.20	13.70	12.50	34.90	0.04	0.00	33.80	56.30	6.20	2.10	0.60	ABSENT	ABSENT	NEGATIVE
12	8802	26/M	3.14	5.51	14.70	41.70	75.70	26.70	35.30	270.00	36.30	13.50	11.20	10.04	27.50	0.28	0.30	73.30	22.60	3.50	0.00	0.00	ABSENT	ABSENT	NEGATIVE
13	8805	24/F	3.76	3.34	8.30	25.30	75.70	24.90	32.80	293.00	59.90	22.00	11.03	10.06	29.70	0.31	0.00	73.90	13.80	10.90	0.30	0.00	ABSENT	ABSENT	NEGATIVE
14	8812	60/M	6.80	4.36	13.40	36.70	84.02	30.70	36.50	138.00	42.30	13.80	12.80	11.30	34.40	0.16	0.00	71.80	14.70	10.40	2.40	0.30	ABSENT	ABSENT	NEGATIVE
15	8815	60/F	7.52	4.81	13.80	38.90	80.90	28.70	35.50	149.00	39.90	13.70	15.10	11.08	34.60	0.18	0.00	79.90	9.20	9.80	0.10	0.30	ABSENT	ABSENT	NEGATIVE
16	8818	47/M	8.93	4.11	12.10	35.80	87.10	29.40	33.80	347.00	41.40	13.20	10.10	9.60	21.10	0.33	0.10	72.80	16.70	4.90	5.00	0.20	ABSENT	ABSENT	NEGATIVE
17	8822	25/M	5.69	5.04	14.20	41.30	81.90	28.20	34.40	110.00	40.20	13.50	12.40	10.40	28.60	0.11	0.00	52.60	31.30	15.30	0.00	0.40	ABSENT	ABSENT	NEGATIVE
18	8824	36./F	22.39	4.22	11.40	33.40	79.10	27.00	34.10	113.00	45.60	15.90	16.20	12.70	43.80	0.14	0.00	95.00	1.80	1.40	0.00	0.30	PRESENT	PRESENT	NEGATIVE
19	8834	38/M	22.04	3.92	10.60	28.10	71.70	27.00	37.70	50.00	38.70	15.20	9.10	9.00	19.50	0.05	0.10	76.10	5.40	5.60	1.10	0.30	ABSENT	ABSENT	NEGATIVE
20	8833	54/M	19.26	2.42	6.20	18.20	75.20	25.60	34.10	193.00	58.60	21.90	8.90	10.00	25.60	0.19	0.40	79.60	4.50	1.60	1.00	0.20	ABSENT	ABSENT	NEGATIVE
21	8865	22/M	2.32	3.40	9.10	28.40	83.50	26.80	32.00	92.00	44.30	14.50	18.50	13.10	48.30	0.12	0.00	49.60	36.60	10.80	0.40	0.00	PRESENT	PRESENT	NEGATIVE
22	8864	60/M	8.93	4.11	12.10	35.80	87.10	29.40	33.80	347.00	41.40	13.20	10.10	9.60	21.10	0.33	0.10	72.80	16.70	4.90	5.00	0.20	ABSENT	ABSENT	NEGATIVE
23	8866	23/M	5.66	5.31	16.00	46.30	87.20	30.10	34.60	262.00	44.10	14.00	14.10	10.90	33.80	0.29	0.20	54.90	30.70	11.10	2.70	0.20	ABSENT	ABSENT	NEGATIVE
24	8867	22/F	8.86	3.83	11.30	35.30	92.20	29.50	32.00	353.00	47.80	14.00	15.60	12.50	46.10	0.44	0.00	64.70	28.60	5.50	0.00	0.10	ABSENT	PRESENT	NEGATIVE
25	8869	63/M	8.24	2.84	8.50	27.30	96.10	29.90	31.10	457.00	63.70	18.30	14.00	11.40	34.90	0.52	0.20	74.80	15.80	4.40	1.20	0.20	ABSENT	ABSENT	NEGATIVE
26	8873	60/F	2.47	2.51	8.70	25.00	99.60	34.70	34.80	7.00	52.50	14.60	0.00	0.00	0.00	0.00	0.40	21.10	70.40	3.60	4.90	0.00	ABSENT	ABSENT	NEGATIVE
27	8876	52/M	9.08	4.03	12.00	34.70	86.10	29.80	34.60	165.00	41.80	13.50	14.10	11.50	34.60	0.19	0.00	75.30	11.70	12.30	0.10	0.00	ABSENT	ABSENT	NEGATIVE
28	8879	20/M	3.90	4.78	15.10	44.40	92.90	31.60	34.00	105.00	42.50	12.30	19.20	13.00	49.10	0.14	0.30	41.40	35.10	17.70	3.80	0.50	ABSENT	PRESENT	NEGATIVE
29	8880	50/F	6.26	3.35	8.70	26.00	77.60	26.00	33.50	16.00	44.10	15.90	18.80	12.00	46.30	0.02	0.20	82.00	11.80	4.20	0.00	0.20	ABSENT	ABSENT	NEGATIVE
30	8881	34/M	5.95	3.57	10.30	30.50	85.40	28.90	33.80	168.00	49.00	15.80	13.50	11.80	34.60	0.20	0.00	64.10	9.60	8.10	17.10	0.30	ABSENT	ABSENT	NEGATIVE
31	8884	48/F	18.69	4.31	14.00	45.30	105.10	32.50	30.90	137.00	91.00	25.70	25.20	14.90	63.30	0.20	1.50	88.80	7.80	1.60	0.00	0.50	PRESENT	PRESENT	NEGATIVE
32	8886	23/F	4.34	4.44	12.70	38.80	87.40	28.60	32.70	195.00	42.40	13.10	25.20	11.80	34.90	0.23	0.00	53.70	35.90	8.80	0.70	0.20	ABSENT	ABSENT	NEGATIVE
33	8887	23/F	2.23	4.02	10.01	33.60	83.60	25.10	30.10	325.00	46.90	15.50	25.20	10.90	30.80	0.35	0.00	71.80	22.90	4.00	0.00	0.00	ABSENT	ABSENT	NEGATIVE
34	8894	36/F	2.61	3.46	8.70	28.40	82.10	25.10	30.60	274.00	56.90	19.10	25.20	13.10	47.20	0.36	0.00	82.50	13.00	3.40	0.00	0.00	PRESENT	PRESENT	NEGATIVE
35	8895	19/M	5.93	5.02	15.50	44.30	88.20	30.90	35.00	179.00	42.50	13.20	25.20	11.60	38.00	0.21	0.00	64.10	18.50	14.50	0.70	0.30	ABSENT	ABSENT	NEGATIVE
36	8896	41/M	14.50	4.71	13.60	37.00	78.60	28.90	36.80	113.00	38.40	13.30	25.20	12.60	46.70	0.14	0.10	88.20	3.90	2.60	0.10	0.30	ABSENT	ABSENT	NEGATIVE
37	8897	60/M	21.50	4.56	13.30	37.70	82.70	29.20	35.30	3.00	47.00	15.60	25.20	8.70	15.60	0.00	0.20	92.10	2.20	1.80	0.10	0.10	ABSENT	ABSENT	NEGATIVE
38	8888	43/F	7.21	3.87	8.20	27.70	71.60	21.20	29.60	207.00	50.00	20.40	25.20	0.00	0.00	0.00	0.10	76.40	15.50	5.10	2.20	0.10	PRESENT	PRESENT	NEGATIVE
39	9116	24/f	3.77	4.58	14.20	42.60	93.00	31.00	33.30	277.00	41.60	12.00	25.20	10.00	25.00	0.28	1.60	44.70	40.80	6.10	7.40	0.50	ABSENT	ABSENT	NEGATIVE

S. NO	ITEM NO.	Age/ Sex	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RDW-SD	RDW CV	PDW	MPV	P CLR	PCT	MRBC	NEUT	LYMPH	MONO	Eosino	BASO	WL FLAG WBC+/- MIXED FLAG	PLATELET FLAG	QBC
40	9123	16/m	6.42	4.85	14.70	38.90	80.20	30.30	37.80	124.00	37.50	13.00	25.20	11.00	32.60	0.14	0.00	76.50	18.80	3.70	0.00	0.20	ABSENT	ABSENT	NEGATIVE
41	9125	17/f	8.01	4.27	11.50	33.60	78.70	26.90	34.20	340.00	44.10	15.50	25.20	9.70	22.40	0.33	0.00	58.50	32.60	7.50	0.70	0.20	ABSENT	ABSENT	NEGATIVE
42	9129	31/m	10.08	5.26	12.10	38.30	72.80	23.00	31.60	267.00	47.30	18.60	25.20	11.20	33.90	0.30	0.00	47.10	25.30	6.90	20.00	0.50	ABSENT	ABSENT	NEGATIVE
43	9131	13/f	4.21	9.09	0.00	77.80	85.60	0.00	0.00	44.00	40.80	16.90	25.20	10.80	33.60	0.05	0.00	48.20	33.50	14.30	2.60	1.20	ABSENT	ABSENT	NEGATIVE
44	9132	31/m	7.41	5.01	15.40	44.90	89.60	30.70	34.30	146.00	38.50	12.10	25.20	13.10	49.90	0.19	0.00	49.80	41.70	7.20	0.70	0.30	PRESENT	PRESENT	NEGATIVE
45	9135	30/m	7.43	4.53	13.30	38.70	85.40	29.40	34.40	54.00	48.50	15.60	25.20	0.00	0.00	0.00	0.00	43.30	36.90	14.00	4.80	0.30	PRESENT	PRESENT	NEGATIVE
46	9142	34/m	4.44	4.69	13.40	37.40	79.70	28.60	35.80	68.00	37.20	13.10	25.20	11.20	32.70	0.08	0.20	60.40	28.20	10.80	0.20	0.20	ABSENT	ABSENT	NEGATIVE
47	9146	35/m	26.87	3.16	10.02	28.40	89.90	32.30	35.90	98.00	45.90	14.30	25.20	13.40	53.60	0.13	0.00	85.90	8.20	3.50	0.10	0.10	PRESENT	PRESENT	NEGATIVE
48	9148	65/f	10.32	3.23	9.20	25.60	79.03	28.50	35.90	95.00	37.50	13.10	25.20	11.80	34.90	0.11	0.00	77.40	11.60	8.40	0.90	0.10	ABSENT	ABSENT	NEGATIVE
49	9415	37/M	10.79	4.71	13.40	38.60	82.00	28.50	34.70	257.00	38.20	12.90	25.20	10.80	30.10	0.28	0.00	60.00	24.20	7.40	7.50	0.30	ABSENT	ABSENT	NEGATIVE
50	9416	20/M	8.42	5.83	17.10	50.30	86.30	29.30	34.00	187.00	42.70	13.70	25.20	10.60	30.70	0.20	0.00	70.00	20.50	9.00	0.10	0.20	ABSENT	ABSENT	NEGATIVE
51	9421	48/F	10.22	4.03	10.40	33.40	82.90	25.80	31.10	278.00	47.40	15.60	25.20	10.50	26.70	0.29	0.00	69.60	22.60	5.30	2.00	0.20	ABSENT	ABSENT	NEGATIVE
52	9427	50/F	7.46	3.27	10.70	29.50	90.20	32.70	36.30	168.00	48.90	14.80	25.20	12.40	34.60	0.21	0.00	75.40	18.50	4.80	0.50	0.10	ABSENT	ABSENT	NEGATIVE
53	9430	21/M	27.00	2.95	8.50	24.70	83.70	28.80	34.40	172.00	51.10	16.70	25.20	11.90	38.60	0.20	0.10	79.90	3.10	5.40	0.00	0.30	ABSENT	ABSENT	NEGATIVE
54	9432	62/F	20.76	2.25	5.40	17.60	78.20	24.00	30.70	402.00	47.20	16.50	25.20	9.90	22.30	0.40	0.00	87.90	6.00	4.80	0.00	0.00	ABSENT	ABSENT	NEGATIVE
55	9434	29/F	7.23	4.23	10.30	31.40	74.20	24.30	32.80	270.00	36.60	13.70	25.20	11.00	31.70	0.30	0.00	48.40	43.30	5.90	1.70	0.30	ABSENT	ABSENT	NEGATIVE
56	9442	52/F	6.23	4.02	7.60	24.70	61.40	18.90	30.80	192.00	43.70	22.10	25.20	0.00	0.00	0.00	1.40	53.50	37.10	6.60	1.60	0.60	PRESENT	PRESENT	NEGATIVE
57	9443	47/F	11.87	4.34	9.60	30.60	70.50	22.10	31.40	395.00	45.20	18.60	25.20	10.70	32.00	0.42	0.20	61.00	20.10	5.20	12.50	0.50	ABSENT	ABSENT	NEGATIVE
58	9445	41/M	7.55	3.87	9.60	31.90	82.40	24.80	30.01	289.00	51.40	17.30	25.20	12.40	41.50	0.36	0.00	92.70	4.50	1.50	0.40	0.10	ABSENT	ABSENT	NEGATIVE
59	9448	59/M	12.39	5.14	13.50	39.60	77.00	26.30	34.10	374.00	39.50	14.20	25.20	9.70	21.50	0.36	0.00	50.80	31.60	10.30	5.20	0.20	ABSENT	ABSENT	NEGATIVE
60	9450	21/M	2.86	4.78	13.60	38.60	80.80	28.50	35.20	142.00	37.80	13.00	25.20	11.70	34.60	0.17	0.30	75.60	17.80	4.90	0.70	0.30	ABSENT	ABSENT	NEGATIVE
61	9452	32/F	1.95	1.81	6.20	18.20	100.60	34.30	34.10	10.00	73.50	22.50	25.20	0.00	0.00	0.00	1.00	57.50	31.30	9.70	0.00	0.50	PRESENT	PRESENT	NEGATIVE
62	9454	18/F	3.47	3.94	12.20	36.20	91.90	31.00	33.70	159.00	43.70	13.00	25.20	11.30	34.20	0.18	0.00	53.30	29.10	16.70	0.30	0.30	ABSENT	ABSENT	NEGATIVE
63	9465	21/M	3.70	4.55	14.10	41.30	90.80	31.00	34.10	175.00	43.80	13.20	25.20	11.10	32.80	0.19	0.30	79.30	11.60	8.40	0.00	0.00	ABSENT	ABSENT	NEGATIVE
64	9467	40/F	8.59	3.81	12.01	37.20	97.60	31.80	32.50	304.00	52.01	14.50	25.20	10.04	27.80	0.32	0.00	57.80	33.60	6.20	2.10	0.10	ABSENT	ABSENT	NEGATIVE
65	9471	20/M	3.90	4.21	11.80	35.30	83.80	28.00	33.40	30.00	44.50	14.60	25.20	9.50	21.00	0.22	0.00	30.00	52.80	15.40	0.80	0.50	PRESENT	PRESENT	NEGATIVE
66	9473	42/M	6.67	3.45	9.60	27.60	80.00	27.80	34.80	104.00	65.90	22.90	25.20	11.10	32.50	0.24	0.10	71.90	12.40	14.20	1.00	0.10	PRESENT	PRESENT	NEGATIVE
67	9474	28/F	6.15	4.64	12.00	36.20	78.00	25.90	33.10	235.00	40.30	14.30	25.20	9.50	21.00	0.22	0.00	63.20	27.00	8.50	0.00	0.20	ABSENT	ABSENT	NEGATIVE
68	9481	19/M	7.90	5.69	17.10	48.60	85.40	30.10	35.20	231.00	40.10	13.20	25.20	10.80	30.90	0.25	0.00	74.10	13.90	11.50	0.00	0.10	ABSENT	ABSENT	NEGATIVE
69	9482	61/F	8.30	4.48	13.00	39.30	87.70	29.00	33.10	324.00	44.80	14.10	25.20	10.70	30.20	0.35	0.00	48.40	39.90	8.10	2.90	0.50	ABSENT	ABSENT	NEGATIVE
70	9483	15/F	6.02	4.26	11.50	34.60	81.20	27.00	33.20	359.00	41.10	13.90	25.20	9.40	19.30	0.34	0.00	47.50	39.90	6.00	5.80	0.50	ABSENT	ABSENT	NEGATIVE
71	9485	19/M	7.55	4.07	10.30	31.50	*77.40*	25.30	32.70	99.00	40.90	15.10	25.20	12.00	37.90	0.12	0.30	17.50	35.60	27.90	12.80	0.80	ABSENT	ABSENT	NEGATIVE
72	9486	23/F	13.61	4.32	13.20	36.10	83.60	30.60	36.60	221.00	38.20	12.50	25.20	11.10	32.50	0.24	0.10	83.00	12.30	4.30	0.10	0.10	ABSENT	ABSENT	NEGATIVE
73	9487	60/F	6.54	2.34	6.20	18.40	78.60	26.50	33.70	182.00	43.30	14.90	25.20	10.80	29.80	0.20	0.00	70.60	17.90	7.60	3.40	0.20	ABSENT	ABSENT	NEGATIVE
74	9490	32/F	3.61	4.33	12.80	36.20	83.60	29.60	35.40	25.00	41.30	13.50	25.20	13.00	45.00	0.03	0.30	32.10	44.90	18.30	2.80	0.80	PRESENT	PRESENT	NEGATIVE
75	9491	48/M	2.69	3.97	12.30	35.00	88.20	31.00	35.10	58.00	52.30	16.40	25.20	11.40	34.70	0.07	0.70	49.50	29.00	16.70	3.70	0.70	ABSENT	ABSENT	NEGATIVE
76	9492	25/M	7.66	2.92	9.60	26.20	89.70	32.90	36.60	68.00	51.50	15.80	25.20	12.60	44.10	0.09	0.40	65.00	20.20	7.30	1.20	0.30	PRESENT	PRESENT	NEGATIVE
77	9495	14/M	10.23	4.43	12.10	35.00	79.00	27.30	34.60	295.00	34.70	12.10	25.20	11.10	33.40	0.33	0.00	45.10	35.10	6.80	12.20	0.30	ABSENT	ABSENT	NEGATIVE
78	9501	26/M	3.51	5.11	14.10	42.30	82.80	27.60	33.30	183.00	41.70	14.00	25.20	11.30	34.60	0.21	0.00	0.00	41.50	42.50	8.30	6.80	ABSENT	ABSENT	NEGATIVE
79	9503	19/M	18.76	5.18	13.90	41.10	79.30	26.80	33.80	271.00	41.60	14.40	25.20	11.20	34.20	0.30	0.00	79.20	9.20	10.60	0.20	0.20	ABSENT	ABSENT	NEGATIVE

S. NO	ITEM NO.	Age/ Sex	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RDW-SD	RDW CV	PDW	MPV	P CLR	PCT	MRBC	NEUT	LYMPH	MONO	Eosino	BASO	WL FLAG WBC+/- MIXED FLAG	PLATELET FLAG	QBC
80	9506	24/F	17.77	4.47	12.00	36.40	81.40	26.80	33.00	273.00	42.20	14.30	25.20	11.10	34.10	0.30	0.00	76.80	11.60	10.50	0.20	0.10	ABSENT	ABSENT	NEGATIVE
81	9512	25/M	4.03	4.64	13.20	38.00	81.90	28.40	34.70	120.00	40.60	13.60	25.20	13.50	52.00	0.16	0.00	49.70	38.00	10.90	0.20	0.20	PRESENT	PRESENT	NEGATIVE
82	9513	36/F	3.51	3.06	9.60	26.50	86.60	31.40	36.20	181.00	46.20	14.60	25.20	10.50	27.10	0.19	0.00	74.30	12.50	6.60	5.40	0.30	ABSENT	ABSENT	NEGATIVE
83	9514	19/M	12.87	3.37	9.50	28.60	84.90	28.20	33.20	176.00	42.60	13.80	25.20	11.00	32.10	0.19	0.10	84.50	7.60	6.60	0.00	0.10	ABSENT	ABSENT	NEGATIVE
84	9811	19/M	4.77	4.17	12.20	35.60	85.40	29.30	34.30	134.00	41.20	13.20	25.20	11.60	34.40	0.16	0.80	68.40	25.80	4.80	0.40	0.20	ABSENT	ABSENT	NEGATIVE
85	9814	19/M	3.45	5.00	15.10	42.20	84.40	30.20	35.80	70.00	36.50	12.00	25.20	11.70	34.90	0.08	0.00	57.40	28.70	11.90	1.40	0.30	ABSENT	ABSENT	NEGATIVE
86	9822	35/F	9.00	3.17	13.90	39.10	123.30	43.80	35.50	318.00	62.60	13.20	25.20	11.00	32.10	0.32	0.20	34.80	46.80	12.40	3.20	0.60	ABSENT	ABSENT	NEGATIVE
87	9823	21/M	6.47	5.31	16.10	48.10	90.60	30.30	33.50	306.00	44.30	13.20	25.20	10.10	24.80	0.31	0.00	68.60	19.50	9.70	1.40	0.20	ABSENT	ABSENT	NEGATIVE
88	9824	32/M	3.72	5.22	13.60	42.50	81.40	26.10	32.00	175.00	42.70	14.60	25.20	11.50	34.60	0.20	0.00	71.00	18.80	9.40	0.00	0.30	ABSENT	ABSENT	NEGATIVE
89	9825	75/M	7.34	3.66	10.30	32.70	89.30	28.10	31.50	201.00	50.10	15.40	25.20	11.30	34.60	0.23	0.00	58.70	29.40	6.50	4.60	0.40	ABSENT	ABSENT	NEGATIVE
90	9826	40/M	13.73	5.74	18.20	53.00	92.30	31.70	34.30	114.00	47.80	14.10	25.20	12.60	44.80	0.14	0.00	78.40	11.90	8.40	0.70	0.10	ABSENT	PRESENT	NEGATIVE
91	9832	57/F	10.28	4.42	12.90	37.50	84.80	29.20	34.40	244.00	40.80	13.10	25.20	10.20	26.90	0.25	0.00	47.50	35.00	8.00	8.90	0.30	ABSENT	ABSENT	NEGATIVE
92	9833	45/M	16.73	3.52	11.60	32.00	90.90	33.00	36.30	229.00	51.80	15.70	25.20	12.00	34.90	0.27	0.10	78.30	10.00	7.90	2.00	0.40	ABSENT	ABSENT	NEGATIVE
93	9836	53/M	10.13	5.31	14.50	43.20	81.40	27.30	33.60	292.00	41.30	14.20	25.20	11.50	34.90	0.33	0.00	55.90	32.00	7.30	4.00	0.30	ABSENT	ABSENT	NEGATIVE
94	9839	45/F	3.76	2.76	8.50	24.20	87.70	30.80	35.10	65.00	55.10	17.40	25.20	12.90	47.70	0.08	0.30	60.10	23.10	13.30	2.70	0.30	PRESENT	PRESENT	NEGATIVE
95	9844	19/F	2.63	0.76	3.20	9.50	125.00	42.10	33.70	65.00	157.50	35.90	25.20	0.00	0.00	0.00	2.70	30.40	58.20	5.70	3.00	0.00	PRESENT	PRESENT	NEGATIVE
96	9846	14/M	11.59	4.68	13.70	36.30	77.60	29.30	37.70	275.00	34.20	12.20	25.20	10.60	29.10	0.29	0.00	83.90	13.20	1.90	0.30	0.20	ABSENT	ABSENT	NEGATIVE
97	9847	74/F	27.91	3.51	10.90	30.60	87.20	31.10	35.60	288.00	45.60	14.30	25.20	10.00	24.60	0.29	0.00	70.60	23.40	5.20	0.00	0.00	ABSENT	ABSENT	NEGATIVE
98	9849	100/F	11.84	4.34	13.70	39.60	91.20	31.60	34.60	71.00	45.30	13.50	25.20	12.30	43.30	0.09	0.10	82.70	10.60	3.90	1.30	0.10	ABSENT	PRESENT	NEGATIVE
99	11654	39/M	1.28	6.04	17.30	52.90	87.60	28.60	32.70	99.00	44.20	14.00	25.20	11.60	34.20	0.11	0.00	39.80	47.70	12.50	0.00	0.00	ABSENT	ABSENT	NEGATIVE
100	11645	65/F	6.93	3.88	11.60	39.90	102.80	29.90	29.10	223.00	51.60	13.70	25.20	13.10	49.70	0.29	0.00	63.50	26.40	7.80	2.00	0.30	PRESENT	PRESENT	NEGATIVE
101	11655	21/M	3.46	5.39	16.10	54.10	104.20	30.80	29.60	118.00	57.19	14.90	25.20	11.50	34.90	0.14	0.00	44.80	43.10	9.80	1.70	0.60	ABSENT	ABSENT	NEGATIVE
102	11665	28/M	6.02	4.02	11.60	34.60	86.10	28.90	38.50	239.00	41.30	13.20	25.20	9.70	20.50	0.23	0.00	81.70	12.50	5.60	0.00	0.20	ABSENT	ABSENT	NEGATIVE
103	11678	38/M	3.59	4.57	12.90	36.70	80.30	28.20	35.10	82.00	40.40	14.00	25.20	13.60	53.50	0.11	0.00	49.20	35.10	14.80	0.60	0.30	PRESENT	PRESENT	NEGATIVE
104	11679	38/M	9.59	3.57	10.10	29.80	83.50	28.30	33.90	69.00	40.40	13.30	25.20	12.70	34.40	0.09	0.20	96.80	0.90	2.10	0.10	0.10	ABSENT	ABSENT	NEGATIVE
105	11693	28/M	10.91	3.20	10.10	30.70	95.90	31.60	32.90	309.00	43.80	12.60	25.20	10.70	29.40	0.33	0.00	82.10	7.00	9.90	0.80	0.20	ABSENT	ABSENT	NEGATIVE
106	11700	24/M	2.50	4.86	14.70	41.40	85.20	30.20	35.50	5.00	42.10	13.50	25.20	0.00	0.00	0.00	0.00	29.20	42.00	28.00	0.00	0.80	PRESENT	PRESENT	NEGATIVE
107	11703	36/M	3.77	5.13	15.00	40.60	79.10	29.20	36.90	27.00	34.70	12.20	25.20	10.30	30.50	0.03	0.00	27.60	56.00	14.30	0.80	1.30	ABSENT	ABSENT	NEGATIVE
108	11706	25/F	2.29	3.24	8.00	24.70	76.20	24.70	32.40	255.00	49.90	18.40	25.20	11.60	34.90	0.30	0.00	75.50	18.80	4.40	1.30	0.00	ABSENT	ABSENT	NEGATIVE
109	11707	48/M	1.74	2.16	7.30	20.00	92.60	33.00	36.50	17.00	71.60	22.20	25.20	0.00	0.00	0.00	2.90	70.10	21.30	0.15	8.60	0.00	PRESENT	PRESENT	NEGATIVE
110	11708	13/F	6.43	4.08	10.90	32.30	79.20	26.70	33.70	221.00	36.40	12.80	25.20	10.80	30.80	0.24	0.00	77.80	11.00	11.00	0.20	0.00	ABSENT	ABSENT	NEGATIVE
111	11709	20/M	3.21	2.85	8.90	25.00	87.70	31.20	35.60	69.00	46.30	14.60	25.20	12.00	34.60	0.08	0.30	35.60	51.70	11.50	0.60	0.60	ABSENT	ABSENT	NEGATIVE
112	11710	58/M	6.44	3.09	9.90	27.60	89.30	32.00	35.90	74.00	54.40	17.10	25.20	12.60	34.80	0.09	0.90	59.50	25.20	13.40	1.60	0.30	ABSENT	ABSENT	NEGATIVE
113	11711	34/F	7.10	3.90	9.90	31.70	81.30	25.40	31.20	245.00	54.00	18.40	25.20	11.30	34.60	0.28	0.10	58.50	31.80	9.60	0.00	0.10	ABSENT	ABSENT	NEGATIVE
114	11767	70/F	4.03	4.64	13.20	38.00	81.90	28.40	34.70	120.00	40.60	13.60	25.20	13.50	52.00	0.16	0.00	49.70	38.00	10.90	0.20	0.20	ABSENT	PRESENT	NEGATIVE
115	11771	57/M	6.26	3.16	9.40	28.50	90.20	29.70	33.00	125.00	54.80	16.90	25.20	9.70	20.60	0.12	0.30	90.30	5.00	4.20	0.30	0.20	ABSENT	ABSENT	NEGATIVE
116	11720	25/M	5.51	5.02	13.90	42.90	85.50	27.70	32.40	237.00	42.10	13.60	25.20	11.20	34.90	0.27	0.00	37.30	48.10	9.80	4.40	0.40	ABSENT	ABSENT	NEGATIVE
117	11721	32/F	4.52	4.71	11.70	36.80	78.10	24.80	31.80	238.00	42.10	14.90	25.20	9.80	22.90	0.23	0.20	46.50	44.90	8.20	0.20	0.20	ABSENT	ABSENT	NEGATIVE
118	11724	35/M	10.33	5.71	16.00	44.80	78.50	28.00	35.70	42.00	37.60	13.30	25.20	12.20	43.90	0.05	0.20	43.10	49.40	6.40	0.30	0.80	PRESENT	PRESENT	NEGATIVE
119	11729	65/F	19.70	2.88	7.90	25.40	88.20	27.40	31.10	353.00	45.80	14.20	25.20	9.80	21.00	0.34	0.00	88.80	6.20	4.90	0.00	0.10	ABSENT	ABSENT	NEGATIVE

MASTER CHART - MALARIA POSITIVE SAMPLES

S. NO	ITEM NO.	Age/ Sex	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RDW-SD	RDW CV	PDW	MPV	P CLR	PCT	MRBC	NEUT	LYMPH	MONO	EO	BASO	per thick	parasitic density	GRADE	SEVERITY	STAGE OF PARASITE	WL FLAG+/- MIXED POPULATION FLAG	QBC	PLATELET FLAG
1	4977	46/m	4.40	3.32	11.00	34.20	103.10	33.10	32.10	33.00	54.80	16.90	22.00	12.60	50.70	0.04	0.00	90.20	5.20	3.90	0.40	0.03	10	4000	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
2	5957	20/m	4.70	3.20	10.80	34.60	108.12	32.00	31.20	36.00	55.10	15.80	21.00	12.80	51.10	0.03	0.00	88.20	6.20	4.90	0.40	0.30	8	3200	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
3	6100	18/m	7.45	3.74	11.20	33.10	88.20	29.50	33.80	20.00	48.30	14.50	22.80	12.50	50.30	0.04	0.10	64.50	24.20	10.10	0.90	0.20	7	2800	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
4	6785	45/m	2.24	4.01	11.20	35.70	89.02	27.93	31.30	29.00	50.60	15.40	17.40	13.30	48.70	0.03	0.30	74.00	12.40	6.30	0.40	0.05	9	3600	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
5	6885	24/m	2.03	4.20	10.80	33.10	78.80	28.70	32.60	33.00	52.40	18.10	17.10	14.20	47.30	0.03	0.10	73.90	12.20	4.50	8.40	0.50	8	3200	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
6	6993	50/m	3.30	2.84	8.10	24.40	85.90	28.50	33.20	28.00	44.60	13.40	15.30	14.00	54.00	0.03	0.30	64.50	19.70	4.10	11.40	0.00	9	3600	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
7	7048	30/f	7.12	3.04	11.00	32.80	107.80	33.70	33.50	24.00	47.80	14.30	22.10	12.90	50.60	0.03	0.10	63.80	24.60	10.30	1.10	0.10	7	2800	2+	MODERATE	ring form+ trophozoite	PRESENT	POSITIVE	PRESENT
8	7066	38/m	3.40	2.61	8.40	24.20	92.70	29.80	34.70	30.00	42.40	13.10	14.50	13.80	53.00	0.03	0.20	63.80	19.70	4.50	12.00	0.00	9	3600	3+	MODERATE	ring form+ trophozoite	PRESENT	POSITIVE	PRESENT
9	7145	32/m	4.20	3.10	11.50	34.10	110.00	29.60	33.70	35.00	53.80	16.10	21.00	13.40	51.40	0.03	0.00	87.20	8.20	3.80	0.50	0.30	9	3600	3+	MODERATE	ring form+ trophozoite	PRESENT	POSITIVE	PRESENT
10	7258	53/m	4.50	3.10	10.70	33.10	106.70	30.90	29.00	38.00	54.10	15.90	21.00	12.50	49.90	0.03	0.00	86.20	9.10	4.00	0.40	0.30	8	3200	3+	MODERATE	ring form+ trophozoite	PRESENT	POSITIVE	PRESENT
11	7312	75/m	7.81	3.83	10.80	32.60	85.10	28.10	33.10	23.00	47.20	13.90	21.70	12.40	51.30	0.04	0.00	62.10	24.70	11.20	1.80	0.20	6	2400	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
12	7360	19/m	7.14	3.21	10.70	32.10	100.00	33.30	33.30	24.00	47.10	14.20	22.30	12.80	50.90	0.03	0.10	69.20	18.80	10.10	1.80	0.00	5	2000	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
13	7362	42/f	2.53	4.24	10.80	33.80	79.71	25.47	31.60	32.00	51.80	16.50	17.20	13.90	47.40	0.02	0.20	73.20	13.20	6.30	6.90	0.00	7	2800	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
14	8911	47/m	2.74	4.31	10.90	34.20	79.35	25.20	32.90	35.00	51.30	16.20	17.10	13.90	47.70	0.02	0.00	82.90	8.70	3.40	2.50	0.30	9	3600	3+	MODERATE	all forms	PRESENT	POSITIVE	PRESENT
15	9346	65/f	2.63	3.10	8.10	22.30	71.90	26.10	31.70	29.00	41.30	14.20	15.30	13.80	53.80	0.03	0.10	65.20	19.80	2.80	10.00	0.10	9	3600	3+	MODERATE	all forms	PRESENT	POSITIVE	PRESENT
16	9280	36/f	2.91	3.20	8.00	23.00	67.60	25.00	34.78	28.00	40.80	13.30	15.40	14.10	55.20	0.02	0.20	64.60	19.70	4.10	11.40	0.00	15	6000	4+	SEVERE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
17	13445	36/m	2.80	3.40	8.20	23.20	68.20	24.10	35.30	27.00	42.40	14.00	15.80	14.10	52.60	0.02	0.00	65.10	22.80	5.10	7.00	0.00	28	11200	4+	SEVERE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
18	14887	62/f	2.40	3.30	7.80	23.10	70.00	23.60	33.80	32.00	40.80	14.80	15.60	13.90	52.80	0.03	0.20	68.10	15.10	7.80	9.50	0.03	9	3600	3+	MODERATE	ring form+ trophozoite	PRESENT	POSITIVE	PRESENT
19	15360	29/M	3.75	5.40	15.70	47.10	87.20	29.10	33.30	97.00	42.60	13.40	16.60	12.40	43.70	0.12	0.30	37.70	46.10	14.90	0.30	0.50	6	2400	2+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
20	16628	20/m	4.01	4.52	11.20	34.80	76.90	24.70	32.20	55.00	36.80	14.30	16.50	13.30	46.10	0.00	0.00	54.20	28.50	12.70	3.60	0.50	8	3200	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
21	17021	24/m	3.94	4.10	10.80	35.80	87.30	23.20	37.90	53.00	37.10	15.20	16.10	13.50	46.20	0.00	0.10	55.20	27.50	13.10	3.20	0.50	6	2400	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
22	15233	40/F	2.44	2.16	5.60	18.50	85.60	26.30	38.50	23.00	55.70	18.20	16.70	13.90	46.80	0.00	0.01	62.80	22.10	8.60	6.10	0.00	10	4000	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
23	15380	13/m	4.73	4.85	13.50	38.10	78.50	27.80	34.80	45.00	41.20	14.30	16.90	13.60	46.60	0.00	0.00	62.40	28.80	7.20	0.80	0.60	9	3600	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
24	15601	51/m	3.21	2.31	6.60	18.10	78.35	28.60	35.50	33.00	37.10	12.90	11.30	10.30	46.00	0.03	0.00	72.40	12.40	6.30	8.40	0.50	38	15200	4+	SEVERE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
25	15606	29/m	4.72	5.79	16.50	48.20	83.20	28.50	34.40	48.00	49.30	16.90	17.30	11.80	44.40	0.06	0.00	61.90	19.90	11.40	5.10	1.30	9	3600	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
26	248	30/f	5.99	4.34	13.60	37.40	86.20	31.30	36.40	28.00	42.50	13.40	24.00	14.20	56.80	0.04	0.01	55.30	29.50	11.20	2.70	0.30	7	2800	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
27	15587	40/m	3.99	4.91	11.50	35.30	71.90	23.40	32.60	66.00	36.80	14.30	0.00	0.00	0.00	0.00	0.00	73.90	12.20	4.50	8.40	0.50	16	6400	4+	SEVERE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
28	18076	23/m	2.99	6.87	10.30	32.40	47.00	15.00	30.20	18.00	48.50	15.10	20.40	13.80	47.60	0.02	0.20	64.80	23.60	10.30	1.10	0.00	7	2800	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
29	15358	40/m	6.48	4.30	10.80	32.90	76.50	25.10	32.80	71.00	41.20	15.30	18.90	13.40	46.80	0.00	0.80	56.80	33.30	8.20	0.90	0.20	0.5	200	2+	MILD	Trophozoite	ABSENT	POSITIVE	PRESENT
30	3210	40/m	6.70	4.17	12.70	36.70	88.00	30.50	34.60	91.00	48.40	13.70	13.00	10.50	29.30	0.10	0.10	55.80	28.20	2.90	3.60	0.00	9	3600	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
31	5427	52/m	6.40	3.29	10.90	31.90	97.00	33.10	34.20	12.00	51.90	14.10	14.50	13.70	38.70	0.00	0.20	73.30	19.60	3.20	3.70	0.00	100	40000	4+	SEVERE	ring form only	PRESENT	POSITIVE	PRESENT

S. NO	ITEM NO.	Age/ Sex	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RDW-SD	RDW CV	PDW	MPV	P CLR	PCT	MRBC	NEUT	LYMPH	MONO	EO	BASO	per thick	parasitic density	GRADE	SEVERITY	STAGE OF PARASITE	WL FLAG+/- MIXED POPULATION FLAG	QBC	PLATELET FLAG
32	5648	71/m	2.90	2.12	5.30	16.80	79.24	27.80	31.00	51.00	52.80	15.70	20.60	13.50	52.80	0.07	0.20	69.30	19.30	5.10	7.00	0.10	8	3200	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
33	673op	20/m	5.10	2.58	8.60	25.00	97.70	38.00	37.00	31.00	70.00	19.80	15.80	11.80	35.90	0.04	0.30	40.20	48.70	8.10	2.70	0.00	8	3200	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
34	6241	18/m	4.56	3.78	11.50	34.40	91.00	30.40	33.40	69.00	53.30	16.10	14.80	12.10	40.00	0.08	0.00	50.80	26.50	16.90	3.90	0.40	7	2800	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
35	6258	54/m	4.79	3.33	9.90	30.20	90.70	29.70	32.80	44.00	52.00	15.80	20.00	14.20	54.50	0.06	0.40	60.80	24.40	9.20	0.20	0.60	8	3200	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
36	6290	24/f	4.34	3.89	6.90	22.80	58.60	17.70	30.30	91.00	38.70	21.30	0.00	13.40	49.80	0.00	0.00	72.00	19.40	6.00	2.10	0.50	7	2800	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
37	6334	62/f	6.36	3.59	10.10	28.50	79.30	28.10	32.40	10.00	53.00	17.20	17.30	11.10	36.50	0.09	0.20	79.60	14.80	3.30	1.10	0.30	7	2800	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
38	6344	22/m	4.11	4.59	12.80	39.00	78.40	27.90	32.80	76.00	48.90	15.90	18.10	12.90	50.10	0.10	0.20	59.20	4.40	0.20	35.50	0.20	0.8	320	2+	MILD	Trophozoite	ABSENT	POSITIVE	PRESENT
39	6478	23/f	2.45	2.75	7.40	21.50	78.10	26.90	32.60	38.00	69.50	23.60	15.70	10.80	34.00	0.04	0.40	33.80	43.30	18.40	4.10	0.00	8	3200	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
40	6813	55/m	4.30	3.75	7.79	23.85	63.80	22.87	29.97	75.00	47.89	16.90	16.90	13.20	55.80	0.03	0.10	73.00	18.40	5.00	3.10	0.40	5	2000	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
41	6981	51/m	3.01	3.34	7.34	23.87	71.46	26.98	31.80	47.00	50.89	16.20	21.80	13.80	54.90	0.06	0.10	67.30	19.30	7.10	7.00	0.10	6	2400	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
42	7171	24/m	4.19	3.49	11.10	33.80	86.50	28.40	32.50	64.00	49.90	16.30	14.50	13.70	38.90	0.06	0.00	48.80	28.50	15.90	4.90	0.40	4	1600	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
43	7630	23/m	6.60	3.51	9.60	28.00	79.70	27.40	33.10	139.00	45.90	14.60	14.00	10.70	32.50	0.15	0.01	73.40	18.30	6.90	1.20	0.10	0.7	280	2+	MILD	Trophozoite	ABSENT	POSITIVE	PRESENT
44	8708	26/f	3.30	2.84	7.90	22.60	79.50	27.80	32.80	27.00	44.20	13.70	15.40	14.20	55.10	0.04	0.30	64.50	19.70	4.20	11.30	0	21	8400	4+	severe	ring form+ trophozoite	PRESENT	POSITIVE	PRESENT
45	4785	36/m	8.74	4.79	13.60	39.90	83.30	28.40	34.10	157.00	42.10	13.80	11.50	10.00	25.10	0.16	0.00	57.20	19.70	15.20	7.10	0.2	0.7	280	2+	MILD	Trophozoite	PRESENT	POSITIVE	PRESENT
46	9114	45/f	5.93	3.58	8.50	27.30	76.30	23.70	31.10	119.00	57.60	21.40	14.60	11.00	33.70	0.13	0.20	52.30	31.00	14.30	1.70	0.2	8	3200	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
47	10315	40/m	9.40	4.91	15.30	43.20	88.00	31.20	35.40	181.00	44.60	13.10	11.80	8.90	23.80	0.12	0.01	55.40	28.20	12.80	3.40	0.1	0.6	240	2+	MILD	Trophozoite	PRESENT	POSITIVE	ABSENT
48	11332	52/m	3.80	4.77	13.00	40.00	83.90	27.30	32.50	114.00	46.30	14.50	12.20	10.10	25.20	0.11	0.00	63.20	24.70	6.10	6.00	0	8	3200	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
49	11352	52/m	8.10	4.81	14.20	41.20	85.70	29.50	34.50	56.00	49.40	15.50	15.20	11.30	31.40	0.15	0.00	60.20	32.80	1.00	6.00	0	7	2800	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
50	11556	45/m	7.60	3.28	9.80	29.30	89.30	29.90	33.40	157.00	53.60	16.50	15.30	10.40	30.70	0.16	0.00	64.20	19.80	10.80	5.20	0	10	4000	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
51	12171	68/m	7.55	3.80	11.60	33.70	88.70	30.50	34.40	19.00	47.30	14.60	1.00	0.00	0.00	0.00	0.10	82.10	7.30	7.70	2.60	0.3	15	6000	4+	SEVERE	all forms	PRESENT	POSITIVE	PRESENT
52	12247	29/m	7.08	3.72	11.00	29.50	79.30	29.60	34.40	81.00	42.00	13.50	17.90	13.30	48.40	0.11	0.10	43.50	39.10	11.60	5.80	0	3	1200	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
53	12299	20/m	4.4	3.84	11.3	35	91.1	29.4	32.3	30	54.5	16.3	23	13.6	50.6	0.04	0	90.2	5.2	3.9	0.5	0.2	11	4400	4+	severe	Trophozoite	PRESENT	POSITIVE	PRESENT
54	12314	40/m	8.06	5.49	13.9	43.2	78.7	25.3	32.2	100	41.1	14.6	13.4	11.3	35.2	0.11	0	65.9	22.2	9.7	1.7	0.5	8	3200	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
55	12415	19/f	6.89	3.99	11.6	38.7	89	28.9	31.6	42	57.9	17.8	22.6	13.1	43.6	0.05	0	50.8	22.9	10.2	13.6	0.2	3	1200	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT
56	12439	28/f	5.60	3.84	11.20	35.10	91.40	29.20	31.90	58.00	56.70	16.90	18.70	11.40	39.80	0.10	0.30	52.40	28.80	10.70	7.30	0.5	7	2800	3+	MODERATE	ring form+ trophozoite	PRESENT	POSITIVE	PRESENT
57	14157	18/m	6.31	4.35	12.1	39.9	91.7	27.8	30.3	33	62.5	18.6	19.6	12.5	46.8	0.04	0	51.8	23.9	16.2	7.6	0.5	6	2400	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
58	14636	18/m	4.74	2.78	9.00	27.10	97.50	32.40	33.20	128.00	52.60	14.90	12.50	11.20	32.80	0.14	0.00	67.90	19.60	9.10	3.20	0.2	9	3600	3+	MODERATE	trophizoite +schizont	PRESENT	POSITIVE	PRESENT
59	64	40/f	6.00	3.64	10.50	31.70	87.10	28.80	33.10	14.00	54.70	17.40	16.10	13.50	47.20	0.00	0.30	72.30	25.80	1.20	0.40	0.2	20	8000	4+	MODERATE	all forms	PRESENT	POSITIVE	PRESENT
60	8147	17/m	3.00	3.64	10.30	29.10	79.90	28.30	33.90	55.00	44.30	13.90	15.70	12.00	41.60	0.07	0.30	44.60	42.30	8.20	4.10	0.5	5	2000	3+	MODERATE	Trophozoite	PRESENT	POSITIVE	PRESENT

S. NO	ITEM NO.	Age/ Sex	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RDW-SD	RDW CV	PDW	MPV	P CLR	PCT	MRBC	NEUT	LYMPH	MONO	Eosino	BASO	WL FLAG WBC+/- MIXED FLAG	PLATELET FLAG	QBC
120	11732	25/M	15..19	3.44	11.50	33.90	98.50	33.40	33.90	154.00	50.90	14.30	25.20	11.20	32.60	0.17	0.10	84.20	9.90	5.50	0.20	0.20	ABSENT	ABSENT	NEGATIVE
121	11733	62/M	13.30	3.46	10.30	28.80	83.20	29.80	35.80	263.00	43.80	14.70	25.20	9.90	23.20	0.26	0.10	65.10	18.80	6.70	9.00	0.40	ABSENT	ABSENT	NEGATIVE
122	11734	42/M	7.05	2.65	9.60	27.00	101.90	36.20	35.60	148.00	77.20	20.60	25.20	10.10	24.70	0.15	0.10	72.50	10.80	15.30	1.10	0.30	ABSENT	ABSENT	NEGATIVE
123	11735	46./F	15.14	2.91	7.00	22.20	76.30	24.10	31.50	354.00	43.70	15.90	25.20	10.80	30.60	0.38	0.00	79.10	11.50	8.20	1.10	0.10	ABSENT	ABSENT	NEGATIVE
124	11737	11/M	11.32	5.12	13.40	41.40	80.90	26.20	32.40	243.00	37.70	13.10	25.20	11.20	34.00	0.27	0.00	57.80	29.90	8.70	3.40	0.20	ABSENT	ABSENT	NEGATIVE
125	11740	20/M	5.64	5.98	17.10	51.80	86.60	28.60	33.00	166.00	41.10	13.10	25.20	11.00	32.70	0.18	0.00	44.40	34.20	15.40	5.50	0.50	ABSENT	ABSENT	NEGATIVE
126	11742	34/M	5.63	5.15	13.90	43.00	83.50	27.00	32.30	189.00	41.80	13.70	25.20	11.20	32.20	0.21	0.00	72.30	11.50	11.50	4.30	0.40	ABSENT	ABSENT	NEGATIVE
127	11743	18/F	5.70	3.72	7.30	26.90	72.30	19.60	27.10	271.00	46.30	17.80	25.20	10.50	29.90	0.28	0.00	56.80	33.70	7.90	1.40	0.20	ABSENT	ABSENT	NEGATIVE
128	11744	18/M	7.50	4.69	14.10	40.60	86.60	30.10	34.70	201.00	41.20	13.30	25.20	9.70	22.01	0.20	0.00	65.80	25.90	7.30	0.70	0.30	ABSENT	ABSENT	NEGATIVE
129	11745	42/M	7.52	5.23	15.60	48.20	92.20	29.80	32.40	282.00	45.10	13.30	25.20	10.30	28.00	0.29	0.00	55.50	33.90	9.80	0.70	0.10	ABSENT	ABSENT	NEGATIVE
130	11746	25/M	10.42	5.35	15.90	46.80	87.50	29.70	34.00	210.00	41.20	13.00	25.20	10.40	28.50	0.22	0.00	68.90	17.40	9.90	3.50	0.30	ABSENT	ABSENT	NEGATIVE
131	11763	13/M	4.62	5.14	13.60	38.60	75.10	26.50	35.20	173.00	35.50	13.30	25.20	11.60	34.90	0.20	0.20	36.20	46.30	17.10	0.20	0.20	ABSENT	ABSENT	NEGATIVE
132	11758	54/M	15.76	3.18	10.30	30.50	95.90	32.40	33.80	393.00	47.90	13.50	25.20	10.50	27.90	0.41	0.00	83.40	6.20	9.70	0.40	0.30	ABSENT	ABSENT	NEGATIVE
133	11761	36/M	6.94	5.13	9.70	33.90	66.10	18.90	28.60	346.00	43.50	19.30	25.20	10.10	28.00	0.35	0.00	83.40	7.30	8.60	0.30	0.40	ABSENT	ABSENT	NEGATIVE
134	11766	24/M	4.89	4.08	11.40	32.70	80.10	27.90	34.90	47.00	40.30	13.80	25.20	13.70	51.20	0.06	0.20	48.30	23.70	7.20	20.00	0.80	PRESENT	PRESENT	NEGATIVE
135	289	20/F	5.46	4.65	12.30	36.40	78.30	26.50	33.80	80.00	45.70	13.10	25.20	11.50	34.60	0.09	0.00	54.40	27.40	13.00	4.40	0.20	ABSENT	ABSENT	NEGATIVE
136	4383	32/F	6.23	5.09	14.60	43.00	84.50	28.70	34.00	158.00	41.50	13.40	25.20	13.20	49.60	0.21	0.00	44.60	44.50	9.60	0.60	0.20	PRESENT	PRESENT	NEGATIVE
137	8833	44/M	18.36	1.25	3.20	9.50	76.00	25.60	33.70	102.00	58.60	21.60	25.20	9.80	23.20	0.10	0.40	79.60	4.20	1.60	0.90	0.20	ABSENT	ABSENT	NEGATIVE
138	9148	47/F	10.32	3.23	9.20	25.60	79.30	28.50	35.90	95.00	37.50	13.10	25.20	11.80	34.60	0.11	0.00	77.40	11.60	8.40	0.90	0.10	ABSENT	ABSENT	NEGATIVE
139	15359	43/M	2.09	3.89	11.10	32.50	83.50	28.50	34.20	49.00	44.10	14.60	25.20	13.90	55.10	0.07	0.50	45.90	40.20	7.70	5.70	0.00	PRESENT	PRESENT	NEGATIVE
140	15361	13/F	1.18	1.30	5.00	14.30	110.00	38.50	35.00	19.00	71.30	18.50	25.20	14.10	55.60	0.00	2.50	14.30	60.20	11.90	0.00	0.00	PRESENT	PRESENT	NEGATIVE